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Phosphate Uptake Rates of Phytoplankton Assemblages Grown at Different Dilution Rates in Semicontinuous Culture[1]

C. A. Suttle and P. J. Harrison

Departments of Botany and Oceanography, University of British Columbia, 6270 University Blvd., Vancouver, B.C. V6T 1W5

Suttle, C. A., and P. J. Harrison. 1986. Phosphate uptake rates of phytoplankton assemblages grown at different dilution rates in semicontinuous culture. Can. J. Fish. Aquat. Sci. 43: 1474–1481.

We studied the effects of pulsed nutrient supplies on the physiology of natural phytoplankton assemblages. A natural phytoplankton community, from an oligotrophic lake, was grown at several dilution rates from 0.1 to $1.5 \cdot d^{-1}$ in P-limited semicontinuous cultures. Particulate C:P and N:P ratios (by atoms) were approximately 300:1 and 50:1, respectively, at the lowest dilution rate and 40:1 and 7:1, respectively, at the highest dilution rate. This demonstrates that a range of P-limited conditions can be established using the semicontinuous culturing technique. The time-course of uptake, in response to a saturating addition of phosphate, revealed that there was a short lag before maximum rates of uptake were observed at all but the highest dilution rate, and that maximum uptake rates over the first 30 min of incubation were higher at intermediate dilution rates. Subsequent to the maximum uptake rate occurring, the relationship between uptake rate and time was species dependent. In *Oscillatoria* sp. dominated cultures the uptake rates declined with increasing cellular P, and the rates of these declines increased with dilution rate; in *Synechococcus* sp. dominated cultures the uptake rates remained constant and were independent of cellular P.

Nous avons étudié les effets d'apports pulsés de produits nutritifs sur la physiologie d'assemblages phytoplanctoniques naturels. On a cultivé une communauté phytoplanctonique naturelle, provenant d'un lac oligotrophe, à divers taux de dilution allant de 0,1 à $1,5 \cdot d^{-1}$, en culture semi-continue, avec limitation de P. Les proportions particulaires C:P et N:P (d'après le nombre d'atomes) étaient d'environ 300:1 et 50:1 respectivement, au taux le plus faible de dilution, et 40:1 et 7:1 respectivement, au taux le plus élevé de dilution. Ceci démontre que l'on peut établir une gamme de conditions de limitation de P, en employant la technique de culture semi-continue. L'évolution de la vitesse d'absorption, en réponse à une addition saturante de phosphates, a révélé qu'il se produisait un court décalage avant l'apparition des vitesses maximum d'absorption, à tous les taux de dilution excepté le plus élevé, et que pendant les 30 premières minutes d'incubation, les vitesses d'absorption maximales étaient plus élevées à des taux intermédiaires de dilution. Après l'apparition de la vitesse maximale d'absorption, la relation entre le taux d'absorption et le temps a montré une variation suivant l'espèce; chez les cultures dominées par *Oscillatoria* sp., les vitesses d'absorption déclinaient avec une augmentation du P cellulaire, et la vitesse de ce déclin augmentait en fonction du taux de dilution; dans les cultures dominées par *Synechococcus* sp., les vitesses d'absorption restaient constantes et ne dépendaient pas du P cellulaire.

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The paradigm that phytoplankton are continuously bathed in a homogeneous dilute solution, in which a single nutrient is limiting, has been replaced by the contemporary viewpoint which envisions potentially limiting nutrients as being supplied in discrete "pulses" of elevated concentration. In recent years, discussion has ensued on the role of such patchiness (e.g. McCarthy and Goldman 1979; Jackson 1980; Turpin et al. 1981; Lehman and Scavia 1982, 1984; Currie 1984), with some of the data for this dialogue arising from experiments on chemostat-grown cultures. In these experiments the chemostat is perturbed with a known concentration of the limiting nutrient. The uptake of the nutrient is then followed, either by its disappearance from solution or by incorporation of isotope into the phytoplankton. This technique has revealed that cells grown in chemostats have specific uptake rates for the limiting nutrient far in excess of maximal growth rates (Conway et al. 1976; Conway and Harrison 1977; McCarthy and Goldman 1979; Goldman and Glibert 1982).

The obvious shortcoming of this approach is that chemostats are not an adequate representation of growth conditions if patchiness is important; by definition, in chemostats cells are being grown under conditions of constant nutrient supply (Rhee 1980). As Quarmby et al. (1982) have shown, the physiological state of phytoplankton in chemostats differs from that of phytoplankton grown in a patchy nutrient environment. As well, ecological studies (Turpin and Harrison 1979; Sommer

[1]This was an invited paper for the P–Plankton Dynamics Symposium at the 27th Conference on Great Lakes Research, International Association for Great Lakes Research, Brock University, St. Catharines, Ont., May 1984.

TABLE 1. Temperatures and concentrations of dissolved and particulate nutrients, at 1 m, in Kennedy Lake on the sampling dates.

	May 15 (Exp I)	August 16 (Exp II)
Temperature (°C)	14	21
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	0.75	—
Soluble reactive P (μM)	<0.05	<0.05
Ammonium (μM)	0.08	<0.04
Nitrate (μM)	0.36	<0.04
Particulate P (μM)	0.05	—
Particulate N (μM)	1.14	0.79
Particulate C (μM)	3.58	12.71

1984) have demonstrated that competitive outcome depends on whether the limiting nutrient concentration fluctuates or is constant.

Clearly, in order to understand processes structuring the aquatic environment, more studies of non-steady-state systems are needed; semicontinuous culturing provides a method to study such systems. Depending on the concentration of the limiting nutrient in the dilution medium, the culture can approximate a chemostat or a batch culture. If the concentration added is low, then the ambient concentration will also be low, and selective pressure will be for high affinity, similar to conditions in a chemostat. Alternatively, if the concentration added is high, then the ambient concentration will fluctuate considerably, and selection will occur initially for high maximum uptake rate, and subsequently for high affinity, similar to conditions encountered in a batch culture.

The purpose of this study was to examine the maximum phosphate uptake rates of natural phytoplankton assemblages, grown at different nutrient supply rates, in phosphorus-limited, semicontinuous cultures.

Materials and Methods

The study site was Kennedy Lake, a medium-sized (64 km^2) oligotrophic lake on Vancouver Island in southwestern British Columbia (49°04′N, 125°30′W). A smaller arm of the lake is fertilized with inorganic nutrients for the purpose of enhancing fish productivity (Stockner 1981), but these additions have little effect in the region of the lake where the samples were collected. Additional limnological features of the lake are described in Stockner et al. (1980).

Sampling and Culturing

Samples (20 L each) were collected at the 1 m depth, 3 km from shore, and gently filtered through 120-μm Nitex® screening to remove large zooplankton. Water was collected for Experiment I (Exp I) on 15 May 1983 and for Experiment II (Exp II) on 16 August 1983. Water temperatures and concentrations of particulate and dissolved nutrients, at the time of collection, are presented in Table 1. After collection, the water was transported to the laboratory, diluted to 40 L with WC medium (Guillard and Lorenzen 1972) as modified for each experiment (see below), and placed in a 20°C incubator at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ continuous irradiance. When the community was in vigorous growth, as determined by increasing in vivo fluorescence, the water was used to initiate the experimental cultures.

The medium used was slightly different for the two experiments. In Exp I, Tris (2-amino-2-hydroxymethyl-1,2 propandiol) and phosphate (K_2HPO_4) were reduced to 0.125 $g \cdot L^{-1}$ and 1.67 μM, respectively, bicarbonate and silicate concentrations were doubled to 300 and 200 μM, respectively, and 98 μM KCl was added to replace the potassium lost when the phosphate concentration was decreased. In Exp II, the medium was further modified by replacing Tris with equimolar MOPS, a buffer which does not interfere with ammonium analysis; phosphate was reduced to 0.50 μM, and 1.0 mM $NaNO_3$ was replaced with 22.5 μM NH_4Cl. Residual ammonium (>0.3 μM) in the cultures, prior to the daily dilutions, confirmed that P was limiting.

Each 1.5-L culture was grown in a Plexiglas® tube (62 mm i.d.) sealed on the bottom with a Nalgene® funnel. A length of silicon tubing was attached to the funnels, through which the cultures were drained. Plexiglas® was not found to decrease the growth rate of two test species, *Asterionella formosa* (clone EvD) and *Chlamydomonas* sp.

Banks of Westinghouse "Super-Hi" Output, Daylight fluorescent bulbs (F72T12/D/SHO) provided illumination from each side. Neutral-density screening was utilized to adjust the photon flux density within the tubes to between 150 and 250 $\mu E \cdot m^{-2} \cdot s^{-1}$ as measured using a quantum meter (Biospherical QSL-100) equipped with a submersible 4π collector. Treatments were randomized within tubes to eliminate light effects; however, at the end of experiments no effect of irradiance was detectable within treatments.

At a set time each day the cultures were thoroughly mixed, and a portion removed; new medium was then added and the cultures mixed again. The amount of culture removed was designed to achieve certain specific growth or dilution rates (D), which were calculated as $-\ln f$, where f is the fraction of the original volume remaining subsequent to a dilution. In Exp I, each of 21 cultures was diluted at one of seven rates (0.10, 0.25, 0.33, 0.50, 0.75, 1.0, and 1.5 $\cdot d^{-1}$), with treatments prescribed in triplicate. In Exp II, the three cultures were diluted at 0.50 $\cdot d^{-1}$.

In vivo fluorescence of cultures was monitored daily and community composition regularly. Cell counts were done using settled samples (Lund et al. 1958), but when small cells were abundant, counts were also made using a haemocytometer. Biomass was estimated from cell volumes, calculated using formulae for similar geometric shapes.

Uptake Experiments

Phosphate uptake experiments were conducted when the community composition was stable, and fluorescence had varied less than 10% for 3 d; cultures were diluted for a minimum of 31 d. In Exp I, uptake experiments were randomized so replicate treatments were not run on the same day; in Exp II, however, the three experiments were run simultaneously. Uptake measurements were not run on all replicate cultures, and data from the 1.0 $\cdot d^{-1}$ treatment were lost because of technical problems. Uptake measurements were made on subsamples taken immediately before the normal dilution time and spiked with phosphate (K_2HPO_4) to a concentration near 9.0 μM. In Exp II, 5.0 μM ammonium was also added, less than 0.5 μM of which was taken up over the course of an experiment. All other nutrients were assumed to be in excess based on their supply ratio in the medium. The disappearance of phosphate was followed using a Technicon® Auto Analyser® and the

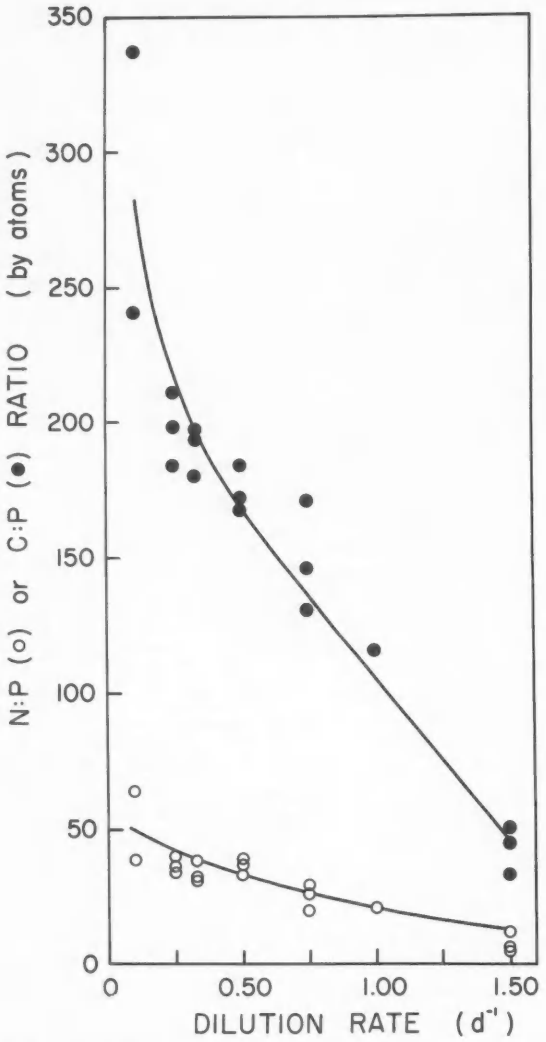

FIG. 1. C:P and N:P ratios (by atoms) for cultures grown at different dilution rates. Each data point represents a single culture. The particulate P concentration is constant and independent of dilution rate. Curves are fitted by eye.

chemical technique of Murphy and Riley (1962) as outlined by Stainton et al. (1977). Following the initial perturbation, phosphate was readded to some samples to maintain a high, saturating concentration. Uptake experiments were carried out at 20°C and 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. Cultures were agitated frequently during experiments. At predetermined time intervals, 15 mL was removed from the subsample and filtered, at a vacuum of less than 120 mmHg, using a Millipore® multiple filtration unit modified to hold larger sample cups. Whatman GF/C filters (mean retention size of 1.2 μm) were used in Exp I; GF/F filters (mean retention size of 0.7 μm) were used in Exp II because of the smaller cell sizes. Filtered standards confirmed that no exchange of phosphate occurred on the filters or filtration apparatus. The filters (24 mm) were pretreated by washing with 20 mL of 3.7% HCl (by wt.), followed by 400 mL of

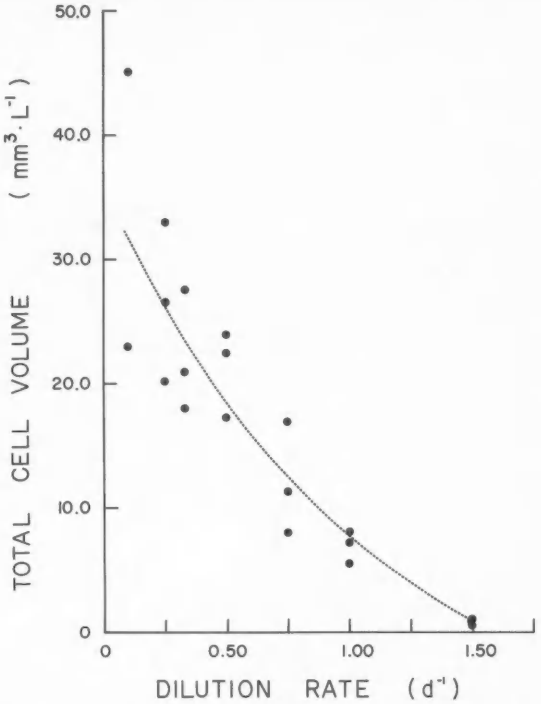

FIG. 2. Total cell volumes for cultures grown at different dilution rates. Each point represents a single culture. Curve is fitted by eye.

deionized, distilled water, and drying at 60°C.

Uptake rates (micromoles per litre per hour) were divided by the initial particulate P concentration (micromoles per litre) to yield nutrient specific uptake rates (V_D^T), where V is the specific uptake rate, T is the interval over which V is calculated, and D is the dilution rate from which the subsample was drawn. This permits an evaluation of uptake relative to cellular requirements, and other authors are urged to provide similar information to allow this calculation. Particulate P was assumed to equal the phosphate concentration in the dilution medium, as soluble reactive P was below detection limits in the culture subsamples. Particulate carbon (PC) and particulate nitrogen (PN) were measured using a Carlo Erba 1106 elemental analyser.

Results

Experiment I: *Oscillatoria*-Dominated Cultures

The carbon to phosphorus (C:P) and nitrogen to phosphorus (N:P) ratios of the phytoplankton (Fig. 1) decrease as D increases. This is consistent with data from P-limited chemostat cultures (Fuhs et al. 1972; Goldman 1979) and shows that daily-dilution, semicontinuous culturing can be used to achieve different degrees of P-limited growth. In a similar manner, biomass (expressed as total cell volume) decreases as D increases (Fig. 2).

The data in Fig. 3 show that cultures diluted at $0.75 \cdot d^{-1}$ and less, demonstrate an acceleration of V from the 1−5 min to the 5−15 min interval (i.e. $V_{0.1-0.75}^{1-5} < V_{0.1-0.75}^{5-15}$), following a phosphate perturbation. After the 5−15 min interval there is a gradual decline in V. This pattern is the same if uptake rate is

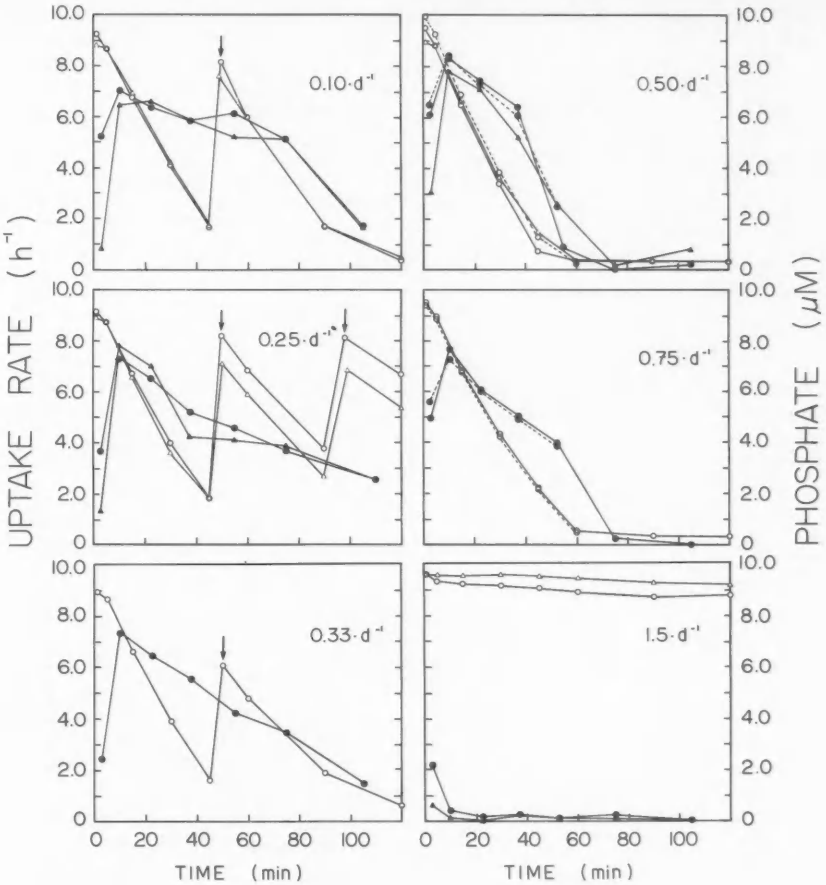

FIG. 3. Changes in perturbation uptake rates, and phosphate concentrations with time, for cultures grown at different dilution rates. Arrows show the first phosphate concentration measured after additional phosphate was added to some cultures. Phosphate concentrations: replicates A (○) and B (△); phosphate uptake rates: replicates A (●) and B (▲). Broken lines join data collected from replicate experiments run on the same cultures on different days.

expressed on a PC basis, as PC changed less than 10%, on average, over the course of the 2-h incubations. It appears that this decline is the result of feedback inhibition and not decreasing substrate concentration, as further addition of phosphate to some cultures (Fig. 3) did not enhance V. Also, experiments conducted on a $0.50 \cdot d^{-1}$ culture filtered through a 3.0-μm filter showed no measurable uptake, indicating that bacteria were not influencing the pattern observed.

The time-courses of V in the $1.5 \cdot d^{-1}$ cultures are strikingly different; $V_{1.5}^{1-5}$ is much higher than in subsequent intervals, but is still much less than $V_{0.1-0.75}^{1-5}$. The difference may be because of the high phosphate supply rate, although community composition may also be important. The $0.1-0.75 \cdot d^{-1}$ cultures were dominated by *Oscillatoria* sp. (93% of total cell volume; range 74–99%) whereas in both $1.5 \cdot d^{-1}$ cultures only 19% of the total cell volume consisted of *Oscillatoria* sp. Small colonial algae, probably Chrysophytes, and a heterotrophic microflagellate, probably *Paraphysomonas* sp., made up most of the remaining cell volume at the $1.5 \cdot d^{-1}$ dilution rate. The community composition of these cultures was likely affected by the presence of the heterotrophic microflagellate, which was present at all dilution rates, but was relatively much more abundant at $1.5 \cdot d^{-1}$.

Figure 4 illustrates that as D increases from 0.10 to $0.50 \cdot d^{-1}$ there is also an increase in V^{5-15} and V^{15-30}. However, the length of time that high V can be sustained decreases as D increases, resulting in a more rapid decline in V with increasing D. Consequently, at later intervals (V^{30-45}, V^{50-60}, and V^{60-90}) the highest V occurs at the lowest D. It is not possible to discern trends in V^{1-5} with D because of the high variability in V^{1-5}. This variability is the result of the error associated with measuring small changes in concentration over short time intervals, and is not physiological variability.

Experiment II: *Synechococcus*-Dominated Cultures

An enhancement of V^{5-15} relative to V^{1-5} was also characteristic of Exp II (Fig. 5). In other respects the results differed from Exp I as a further increase in V was observed in the interval subsequent to V^{5-15}, and V remained higher for a

longer period. Unlike Exp I, these cultures were dominated by a chroococcoid cyanobacterium, probably *Synechococcus* sp.; no other cell types were observed to be present. This is consistent with the view that cells with high surface area to volume ratios should be superior nutrient competitors (Smith and Kalff 1982, 1983). In Exp II, cells were counted at the beginning and the end of the uptake period; however, no change in numbers was discernible.

Discussion

We made three major observations. Firstly, there was a delay before the maximum uptake rate (V_{max}) occurred, subsequent to phosphate addition to P-limited cultures. Secondly, the highest uptake rates over the first 30 min of incubation were associated with intermediate dilution rates. Thirdly, the manner in which V changed, following a phosphate addition, appears to be species specific.

Delayed Maximum Uptake Rate (V_{max})

The observation of an increasing uptake rate subsequent to a nutrient pulse has been observed for nitrate- (e.g. Collos 1980; Dortch et al. 1982; Parslow et al. 1984a) and silicate-starved (Conway and Harrison 1977) cultures, but has seldom been reported in response to phosphate addition. Chisholm and Stross (1976a) found such a delay when phosphate was added to nutrient-replete cultures of *Euglena gracilis*, which were synchronized to a 14 h light : 10 h dark cycle. The time scale of the response (2 h) was inconsistent with our results; as well, the uptake rate was constant when P limitation was also imposed on the synchronized division cycles (Chisholm and Stross 1976b). Similarly, perturbations of P-limited continuous cultures (Burmaster and Chisholm 1979; Gotham and Rhee 1981) and P-starved batch cultures (Simonis et al. 1974; Brown et al. 1978; Senft et al. 1981; Parslow et al. 1984a) did not result in lags before V_{max} occurred.

It is not clear why the conditions of this study permitted the observation of an increase in saturated uptake rates with time in the initial intervals following a phosphate addition, while experiments where cells were starved and then pulsed with phosphate have not. The most reasonable explanation seems to be that V_{max} is in some way tied to the cell cycle, and that repeated pulsing is required to entrain the cells. This may reveal cellular processes which are normally obscured in a randomly dividing population. Although there was no evidence of an increase in cell number over the course of the uptake experiments, V_{max} could be out of phase, but associated with synchronous division outside of the uptake period. The absence of a delay in V_{max} in the cultures diluted at $1.5 \cdot d^{-1}$ is consistent with this reasoning, as cells whose average generation times are shorter than 1 d are unable to entrain to a rhythm with a circadian periodicity (Chisholm 1981). Cell division in phytoplankton can be entrained to N pulses (Yoder et al. 1982; Chisholm et al. 1984) and Si pulses (Busby and Lewin 1967; Sullivan 1977) but to our knowledge has not been reported for P pulses. In contrast, liquid cultures of higher plant cells can be entrained to phosphate pulses (Amino et al. 1983); consequently, phytoplankton may respond similarly under certain conditions.

The observation of enhanced uptake capability for phosphate when cells are grown under phosphate-limited conditions is well described for bacteria (e.g. Rosenberg et al. 1969), cyanobacteria (e.g. Falkner et al. 1980, 1984; Riegman and Mur 1984), fungi (e.g. Burns and Beever 1979), and microalgae (for review see Cembella et al. 1984). This could be an adaptation to take advantage of ephemeral, elevated concentrations (sensu: McCarthy and Goldman 1979), or a mechanism to increase growth rates at low homogeneous concentrations (Parslow et al. 1984a, 1984b). It is fairly well agreed (cf. Burns and Beever 1979; Gotham and Rhee 1981) that the high-capacity uptake system is regulated by being derepressed when phosphate is limiting to growth. Our data do not contradict this

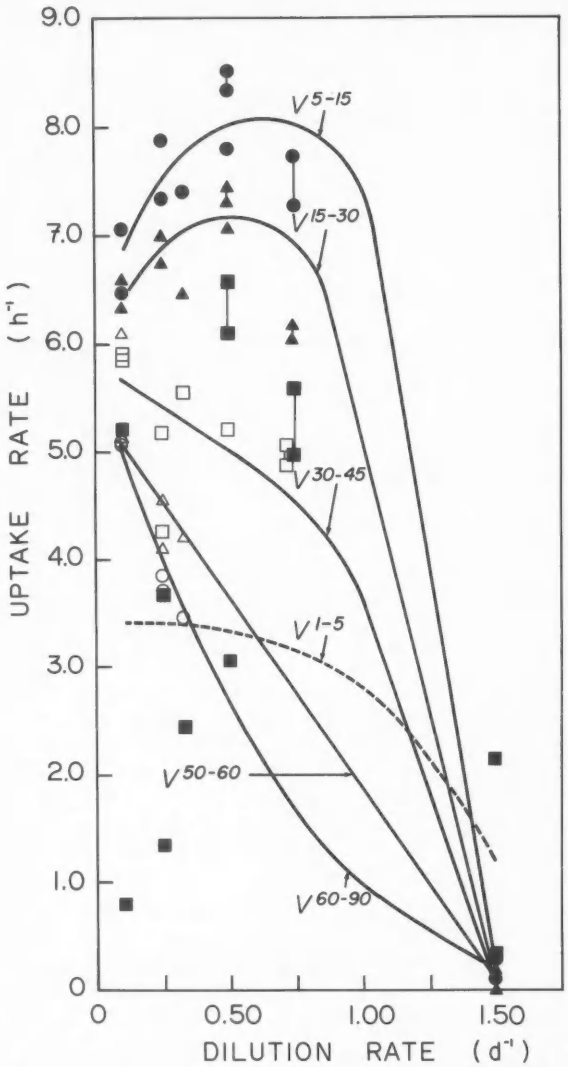

FIG. 4. Perturbation uptake rates for different uptake intervals and dilution rates. Only rates over intervals where the phosphate concentration remained above 1.5 μM are shown. Vertical lines join data collected from replicate experiments run on the same cultures on different days. ■, V^{1-5}; ●, V^{5-15}; ▲, V^{15-30}; □, V^{30-45}; △, V^{50-60}; ○, V^{60-90}. The broken line fitted for V^{1-5} represents a conservative interpretation and is not meant to imply a specific relationship. Curves are fitted by eye.

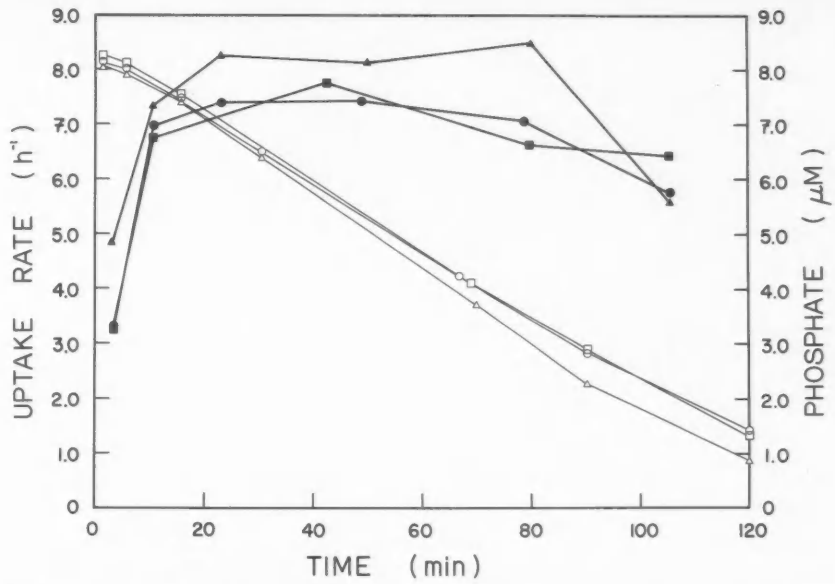

FIG. 5. Perturbation uptake rates and phosphate concentrations for *Synechococcus*-dominated cultures grown at $0.50 \cdot d^{-1}$. Phosphate concentrations: replicates A (○), B (△), and C (□); phosphate uptake rates: replicates A (●), B (▲), and C (■).

model, but suggest that under the experimental conditions we used, an allosteric activator may also be important. This activator would increase the affinity of the transport system for phosphate and be responsible for the enhancement of uptake from V^{1-5} to V^{5-15}. The time-scale of the enhancement may indicate that the formation of the activator is associated with filling an internal pool, as opposed to de novo protein synthesis.

A final point is that although V^{5-15} is, on average, enhanced threefold over V^{1-5}, the uptake rate in the initial period is not low. V^{1-5} is enhanced at least 100 times more than required to sustain the specific growth rate averaged over 24 h, except in those cultures diluted at $1.5 \cdot d^{-1}$.

Changes in Uptake Rate (V) with Dilution rate (D)

Although the culturing technique was able to generate a variety of P-limited states, as shown by the range of C:P and N:P ratios observed, the uptake pattern was not consistent with much of the data obtained from chemostats and batch cultures. In this study the highest uptake rates over the 30 min following a phosphate perturbation are associated with cultures maintained at intermediate dilution rates (Fig. 4). Standardizing the data to PC or cell volume would only exaggerate this relationship because of the high values of these parameters at low dilution rates (Fig. 1 and 2). Although the highest uptake rates have occasionally been found to be associated with intermediate nutrient supply rates in phytoplankton (Burmaster and Chisholm 1979; Healey 1985) and higher plants (Schjørring and Jensen 1984), they are typically associated with the lowest dilution rates (Rhee 1973; Gotham and Rhee 1981; Falkner et al. 1984; Riegman and Mur 1984) at which cell viability is retained (Rhee 1980). (Although the uptake rates in these studies are expressed on a per cell or per biomass basis, the same would be true for specific uptake rates, as cell quota decreases as D decreases.) Similarly, in batch culture the specific uptake rate (Parslow et al. 1984a), the uptake rate per cell (Aitchison and Butt 1973), and the uptake rate per chlorophyll *a* (Falkner et al. 1984) typically increase with increasing phosphate starvation. However, maximum uptake rates have also been found to be constant over a range of dilution rates when expressed on a biomass basis (Healey and Hendzel 1975; Nyholm 1977), and over a range of starvation times when expressed on a per cell basis (Parslow et al. 1984a).

Sustainability of High Uptake Rates (V)

The duration that high specific uptake rates can be sustained differs between Exp I and Exp II. In the *Oscillatoria*-dominated cultures of Exp I, V declined with each successive interval from V^{5-15} (Fig. 3), with the rate of this decline increasing with D (Fig. 4). Parslow et al. (1984a) also found that V declined rapidly following a phosphate spike to starved cells. Declines in V with short-term accumulation of cellular P suggest that phosphate uptake in *Oscillatoria* sp. is regulated by feedback on the uptake system by an internal P pool, as has been proposed for seven species of cyanobacteria (Riegman and Mur 1984; Riegman 1985). Our data indicate that such an internal pool is not directly coupled to the total P content of the cells, as V initially increases and then decreases as cellular P rises.

In the *Synechococcus*-dominated cultures of Exp II there was no reduction in V (Fig. 5), or in the uptake rate per cell, in response to increasing cellular P over the short term. This pattern of a sustainable high uptake rate per cell, while cell quota is rapidly increasing, has also been observed for *Monochrysis lutheri* (Burmaster and Chisholm 1979).

The ability to sustain high uptake rates for an extended period would be of competitive importance in areas where ephemeral pulses of elevated nutrient concentration exist. As yet, patches of nutrients saturating to uptake have not been demonstrated to exist for sufficient time to have a selective

role, although solitons (Holligan et al. 1985) may provide such a mechanism.

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Does Orthophosphate Uptake Supply Sufficient Phosphorus to Phytoplankton to Sustain Their Growth?[1,2]

David J. Currie[3]

Département de Sciences biologiques, Université de Montréal, C.P. 6128, Succursale "A", Montréal, Qué. H3C 3J7

Currie, D. J. 1986. Does orthophosphate uptake supply sufficient phosphorus to phytoplankton to sustain their growth? Can. J. Fish. Aquat. Sci. 43: 1482–1487.

It is generally supposed that phytoplankton obtain their P predominantly from orthophosphate uptake. Yet, phytoplankton are often observed to be responsible for only a very small fraction of the total planktonic community uptake of orthophosphate. Is this uptake of orthophosphate by algae nonetheless sufficient to meet their metabolic needs? To answer this question, I have compared the specific doubling rates of C and of P through orthophosphate uptake in the phytoplankton community of Lake Memphremagog. Orthophosphate uptake during the growing season supplied only a very small portion of the P required to support algal growth. Presumably, the phytoplankton obtained their P from some source other than orthophosphate uptake. Similar patterns of orthophosphate uptake in other P-limited lakes suggest that this phenomenon may be widespread.

Il est généralement supposé que le phytoplancton des eaux douces acquiert du P principalement au moyen de l'absorption de l'orthophosphate dissous. Cependant, plusieurs études déjà publiées indiquent que le phytoplancton n'est généralement responsable que d'une très petite partie de l'absorption totale d'orthophosphate par la communauté planctonique. Cette faible absorption fournit-elle néanmoins suffisamment de P pour les exigences métaboliques du phytoplancton? Afin de répondre à cette question, nous avons comparé le taux de doublage spécifique de C et celui de P par l'absorption d'orthophosphate dans le plancton du lac Memphrémagog. Durant la saison estivale, l'absorption de l'orthophosphate n'a fournit qu'une très petite fraction du P nécessaire pour la croissance algale. Vraisemblablement, le phytoplancton a obtenu son P d'une source autre que l'absorption d'orthophosphate. Une similarité dans les patrons d'absorption d'orthophosphate dans d'autres lacs où le P limite la croissance algale suggèrent que ce phénomène pourrait être général.

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The goal of ecology is, arguably, to predict the abundance and kinds of organisms in nature (cf. Peters 1980; Rigler 1982). The freshwater phytoplankton are of particular interest in this respect, both because the dynamics of many other groups of freshwater organisms depend upon the phytoplankton (e.g. Makarewicz and Likens 1979; McCauley and Kalff 1981; Cole 1982) and because the quality of freshwater from a human perspective depends to a considerable extent upon the abundance and the kinds of phytoplankton present (Vallentyne 1974). The total biomass of phytoplankton in freshwater can be predicted, at least approximately, as a function of the total amount of P present (Dillon and Rigler 1974; Oglesby and Schaffner 1978). However, the kinds of phytoplankton that will be present has proven to be a more elusive problem. A number of models to explain phytoplankton community composition have been considered (e.g. O'Brien 1974; Tilman 1981; Holm and Armstrong 1981; Smith and Kalff 1983; Kilham 1984). A nearly universal feature of these models is the postulate that the growth and dynamics of each individual species are explicit functions of utilization of ambient orthophosphate. More broadly, the fundamental justification of a great variety of studies of P dynamics in freshwater hinges on the supposition that orthophosphate uptake drives phytoplankton growth.

In contrast, several studies have suggested that bacterioplankton dominate orthophosphate dynamics in freshwater (Rigler 1956; Lean and White 1983; Currie and Kalff 1984b). Currie et al. (1986) argued that nearly all the extant evidence indicates that less than 6% of the orthophosphate uptake by the plankton community can be traced to phytoplankton cells under P-deficient conditions (but cf. Paerl and Lean 1976; Levine et al. 1986). If phytoplankton acquire so little of the orthophosphate, one must inquire if this uptake can supply sufficient P to meet the needs of phytoplankton growth. If not, then it no longer seems reasonable to suppose that phytoplankton dynamics are a direct function of orthophosphate utilization.

The purpose of the present study was to test the hypothesis that orthophosphate uptake constitutes the principal source of P for phytoplankton growth. This was tested by comparing the rate at which phytoplankton double their P via orthophosphate uptake with an estimate of their growth rate: the doubling of cellular C. It is evident that, during balanced growth, cells must double essential constituent elements (e.g. P) at least as quickly

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[2] A contribution to the Centre de Recherches Écologiques de Montréal.

[3] Present address: Department of Biology, University of Ottawa, 30 Somerset St. E., Ottawa, Ont. K1N 6N5.

as the cells themselves double. If a gross discrepancy between uptake and growth were observed, then the organisms in question must either draw upon intracellular stores or take up some other form of P.

Methods

A series of experiments was carried out in Lake Memphremagog, a large, hardwater, oligomesotrophic lake 100 km southeast of Montreal, Que. (Ross and Kalff 1975). In the area sampled (Quinn Bay), one usually observes summertime chlorophyll *a* concentrations of 4–7 $\mu g \cdot L^{-1}$, bacterial abundances (DAPI total counts) of approximately 5×10^9 cells$\cdot L^{-1}$ (D. Bird, McGill University, unpubl. data), and total P concentrations of 6–12 $\mu g \cdot L^{-1}$ (D. J. Currie, unpubl. data). At six dates during the ice-free season, integrated samples over the depth of the epilimnion were taken using a length of Tygon tubing. The samples were transported to a lakeside laboratory where experiments were begun immediately to measure the doubling rates of P and of C in the plankton community.

P Doubling Rate via Orthophosphate Uptake

The P doubling rate was estimated from the specific rate of orthophosphate uptake, that is, the gross uptake of P as orthophosphate per unit time, per unit P in biomass. This yields units of doublings of P per day. It is calculated as the product of the rate constant of [^{32}P]orthophosphate uptake, times the ambient orthophosphate concentration, divided by the particulate P concentration. This calculation assumes that orthophosphate uptake is a first-order process, which appears to be very nearly true at low orthophosphate concentrations (Rhee 1973; Tilman and Kilham 1976; Burmaster and Chisholm 1979).

The rate constant of uptake is the proportion of the ambient orthophosphate taken up per unit time. It was estimated by adding carrier-free [^{32}P]orthophosphate to triplicate 50-mL samples of whole lake water and rapidly agitating. At intervals from 20 s to 30 min after addition of the isotope, 2.0-mL aliquots were withdrawn from each flask and were filtered through parallel 0.2- and 3.0-μm Nuclepore polycarbonate filters (the choice of pore size is explained below). The filters were then assayed by liquid scintillation counting in a high-efficiency fluor. The resulting data represent the percent of the [^{32}P]orthophosphate incorporated into particles as a function of time. The rate constant of uptake is equal to the initial slope of this function. It was found by fitting a second-degree polynomial to the observations and then evaluating the first derivative at time zero (Currie and Kalff 1984b). This is equal to the coefficient of the linear term of the polynomial. The standard error of the rate constant of uptake was therefore taken as the standard error of the linear term.

The P in biomass was estimated from the quantity of P retained by the 0.2- and the 3.0-μm filters. It was measured as the difference between the total lake water P and the filtrate of these filters (Menzel and Corwin 1965). This assumes that all P larger than 0.2 μm is in living biomass and that P in detritus is negligible.

Orthophosphate Concentration: Rigler's Bioassay

The ambient orthophosphate concentration was estimated by Rigler's (1966) bioassay. Rigler's method is designed specifically to measure orthophosphate, as opposed to soluble molybdenum-reactive phosphorus (SRP), the latter usually being much higher than the former in oligotrophic waters (Rigler 1966, 1968; Peters 1979; Tarapchak and Rubitschun 1981). A difficulty with this bioassay is the subjectivity of its endpoint. One must judge which of several mathematically undefined, curvilinear functions is most likely to extrapolate to the origin (Rigler 1966). To overcome this difficulty, I have adopted a defined convention. Virtually all extant evidence indicates that orthophosphate uptake by planktonic organisms is a monotonic positive, decelerating function of orthophosphate concentration (e.g. Rhee 1973; Tilman and Kilham 1976; Burmaster and Chisholm 1979). This function should therefore have no inflection points. In Rigler's method, as one assumes higher orthophosphate concentrations, an inflection point appears in the curve (Fig. 1). One unambiguous upper limit to the orthophosphate concentration is the concentration which, if exceeded, will cause the uptake function to develop an upward concavity. Operationally, this concentration may be found by ordering the observations from lowest to highest and estimating the slopes of rectilinear line segments connecting adjacent points. For each adjacent pair of line segments, there is an assumed P concentration at which the slopes become equal. At concentrations higher than this, the curve becomes locally concave upward. Therefore, this P concentration is the highest at which there is no inflection point in the part of the curve defined by these two line segments. This process is repeated for each adjacent pair of line segments. The lowest resulting P concentration represents the highest P concentration at which the curve as a whole is uniformly concave downward (i.e. has no inflection points; Fig. 1). Mathematically, this concentration P may be found from

$$P = \min_{3 \le i \le n} \left[\frac{B(k_i x_i - k_{i-1} x_{i-1}) - k_{i-1} x_{i-1} + k_{i-2} x_{i-2}}{k_{i-1} - k_i - B(k_i - k_{i-1})} \right]$$

where x_1 to x_n represent the n added orthophosphate concentrations used in Rigler's procedure, arranged such that

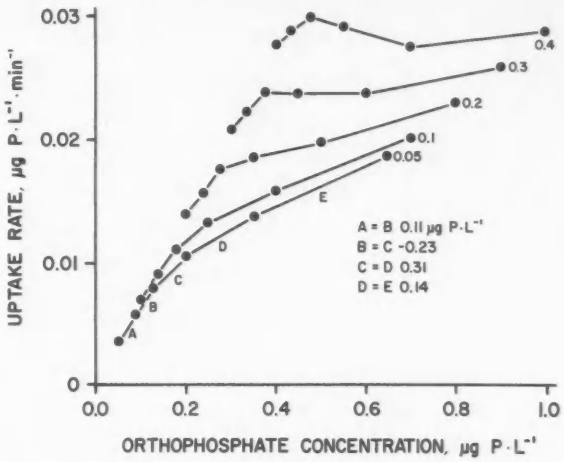

FIG. 1. Example of a Rigler bioassay (24 August 1984). Each curve is labelled on the upper right with the assumed ambient orthophosphate-P concentration. As higher concentrations are assumed, the curve develops upward concavities, first at section A–B and then at sections D–E. The concentrations at which the slopes of adjacent line segments (A–E) become equal are given on the lower right. The Rigler estimate of orthophosphate-P concentration in this case was taken as 0.11 $\mu g \cdot L^{-1}$.

TABLE 1. Percentage of glucose (GLU), bicarbonate (BICARB), and orthophosphate (PO_4) uptake associated with particles larger than 3.0 μm. Also given are the uptake rate constants into particles larger than 0.2 μm ($k_{0.2}$) and 3.0 μm ($k_{3.0}$) (±1.0 SE; $n \geq 15$), the ambient orthophosphate concentration [PO_4] (Rigler upper limit), and the particulate P larger than 3.0 μm [PP] (±1.0 SE; $n = 4$).

Date	% uptake > 3.0 μm			$k_{0.2}$ (d^{-1})	$k_{3.0}$ (d^{-1})	[PO_4] (nM)	[PP] (nM)
	GLU	BICARB	PO_4				
17 May 1984	16.1	100	16.2	38.0 (±4.6)	6.17 (±0.32)	0.34	171 (±3)
6 July 1983	9.1	97.9	4.8	119.2 (±10.3)	5.68 (±0.58)	1.1	190 (±1)
14 July 1984	13.1	78.6	3.2	106.5 (±7.0)	3.44 (±0.22)	0.37	112 (±5)
3 Aug. 1984	21.8	77.1	10.0	139.5 (±14.6)	14.0 (±1.9)	0.42	127 (±2)
24 Aug. 1984	23.4	93.8	25.9	93.1 (±4.6)	14.6 (±1.3)	3.6	94 (±3)
21 Sept. 1984	28.7	81.1	13.8	85.7 (±12.4)	11.9 (±1.1)	—[a]	126 (±9)

[a] Missing datum.

$x_1 < x_2 < \ldots < x_n$; k_i is the uptake rate constant at orthophosphate concentration x_i; and $B = (x_{i-1} - x_{i-2})(x_i - x_{i-1})^{-1}$. Negative values for the P concentration can result from this process. I interpret this to mean that no assumed positive P concentration can cause the curve to develop an inflection point in the segment concerned, and therefore the segment bears no information on the measure of orthophosphate. Therefore, negative concentration estimates were simply ignored.

C Doubling

The C doubling rate was estimated from the primary production rate divided by the algal particulate carbon. Production of particulate C was estimated by the ^{14}C method (Wetzel and Likens 1979). Light and dark bottles were inoculated with [^{14}C]bicarbonate, incubated in situ at several depths, and the accumulation of label in particulate matter was determined. These measurements were integrated over the depth of the epilimnion and over the length of the daylight period (assuming that photosynthesis is proportional to insolation). Algal particulate C was estimated from algal cell volume, determined microscopically by the Utermöhl technique and converted to C according to Mullin et al. (1966).

To be sure, the ^{14}C method is subject to a number of difficulties which can potentially introduce both positive and negative errors (Peterson 1980; Carpenter and Lively 1980). Nonetheless, one may ask the brute empirical question: in practice, how close does the method come to true productivity? Several recent studies indicate that the ^{14}C results are in fact reasonably close to net productivity in relatively oligotrophic systems, diverse sources of error notwithstanding (Jackson 1983; Smith et al. 1984; Platt et al. 1984). I chose to use this technique because it appears to be the best method available to measure in situ productivity, as well as being reasonably accurate.

In order to distinguish algal and bacterial uptake of orthophosphate, uptake by two size classes of particles was partitioned: particles retained on 0.2-μm pore diameter Nuclepore filters and particles retained on 3.0-μm filters. The 0.2-μm filters retain both bacteria and algae (D. J. Currie and D. Bird, unpubl. data). The 3.0-μm filters retain all phytoplankton except some of the smallest picoplankton. They also retain some bacteria. To assess the efficiency of algal–bacterial separation by this procedure, I similarly size-fractionated [^{14}C]glucose uptake (a marker of bacterial activity) and the uptake of [^{14}C]bicarbonate in the light (a marker of algal activity) (Currie and Kalff 1984b). Although certain algae can take up glucose, the rates are very much slower than those of bacteria at low glucose concentrations (Wright and Hobbie 1966; Bonin and Maestrini 1981).

Results

The basic results of the size fractionation experiments are presented in Table 1. It is apparent that, in Lake Memphremagog, 3.0-μm filters retained most algae and relatively few of the bacteria. As in earlier work (Currie and Kalff 1984b; Currie et al. 1986), only a small portion of the orthophosphate uptake was associated with this fraction. Given this observation, one may now inquire whether this orthophosphate uptake is sufficient to support algal growth. I shall approach this question by two paths.

First, consider the rate at which particles larger than 3.0 μm double their P content by orthophosphate uptake. Note that this size fraction necessarily includes nearly all phytoplankton except the smallest picoplankton. The P doubling rate of this fraction is compared with the rate at which phytoplankton doubled their C in Table 2. On four of the five occasions for which data are available, particles of this size were doubling their C more than an order of magnitude more rapidly than orthophosphate uptake would double their P.

In fact, this approach overestimates the uptake of P by algae. Rigler's bioassay provides an upper limit to the orthophosphate concentration (Rigler 1966), so that the gross P uptake rate was less than that estimated here. Also, the presence of bacteria in the fraction larger than 3.0 μm (Table 1) serves to inflate the uptake of orthophosphate per unit P, because bacteria have much higher specific uptake rates than do algae (Currie and Kalff 1984a). As a result, the average rate at which algae in this size fraction double their P is less than the measured rate (i.e. the average rate for all particles retained in 3.0-μm filters) given in Table 2. I conclude that, except for one occasion at the

TABLE 2. Estimated doubling rate of P based on orthophosphate uptake and that of C in particles larger than 3.0 μm (±1.0 SE). Also given are the percentage of orthophosphate uptake associated with algae (regardless of size) (%) and the growth rate that this uptake would support (μ, in doublings·d^{-1}).

	Doublings·d^{-1} > 3.0 μm		P uptake by algae	
Date	P	C	%	μ
17 May 1984	0.012 (±0.002)	0.68 (±0.11)	0.12 (±0.02)	0.01 (±0.01)
6 July 1983	0.033 (±0.005)	0.70 (±0.15)	0	0
14 July 1984	0.011 (±0.003)	0.57 (±0.14)	0	0
5 Aug. 1984	0.046 (±0.009)	1.15 (±0.25)	0	0
24 Aug. 1984	0.560 (±0.059)	0.20 (±0.02)	3.6 (±0.4)	0.13 (±0.08)
21 Sept. 1984	—[a]	—[a]	0	0

[a] Missing datum.

end of the growing season, the uptake of orthophosphate from solution was supplying a negligible fraction of the P needed for the growth of organisms larger than 3.0 μm.

In a second approach, the information in Table 1 permits one to calculate explicitly the percentage of the total orthophosphate uptake for which algae, regardless of size, are responsible, and the doubling rate that this would support. The calculation assumes that bacteria in the size fraction larger than 3.0 μm take up glucose and orthophosphate in the same ratio as do bacteria in the smaller size fraction. It also assumes that phytoplankton in the two size fractions take up bicarbonate and orthophosphate in the same ratio. Based on these assumptions, one may write a pair of simultaneous equations to solve separately for algal uptake and for bacterial uptake (Currie and Kalff 1984b). This calculation is necessarily less precise than the one above, since it requires considerably more arithmetic manipulation of parameters measured with error. On four of the six dates sampled, orthophosphate uptake into the plankton community was estimated to be entirely bacterial (Table 2). On the remaining two occasions, phytoplankton were responsible for less than 4% of the total uptake. This uptake of P would have permitted doubling rates of 0–0.01 divisions per day on all dates sampled except 24 August 1984, when 0.13 divisions would have been possible. In comparison, phytoplankton were growing at 0.20–1.2 divisions per day, based on carbon doubling. Thus, the uptake of P as orthophosphate by the phytoplankton community as a whole was small to negligible, relative to the quantity required to sustain balanced growth.

Discussion

The fundamental observation of this study is that phytoplankton doubled their P by orthophosphate uptake much more slowly than they doubled their C. From this observation, I conclude that phytoplankton, particularly those larger than 3.0 μm, must have obtained negligible P from orthophosphate, based on the following reasoning. My six experiments each showed negligible instantaneous P doubling rates by algae, based on size fractionation. A much more extensive series of fractionation experiments showed exactly the same fractionation patterns diurnally as well as seasonally from May to October (Currie and Kalff 1984b; Currie et al. 1986). Balanced growth is therefore ruled out for the entire summer. In principle, phytoplankton could have grown using stored P taken up before or after this period. However, in an earlier study, Sproule and Kalff (1978) found very little surplus P (boiling water extractable P) in plankton from this part of Memphremagog between May and October. Furthermore, it seems highly unlikely that species which are absent in early spring and which wax later in the season could grow using orthophosphate taken up before mid-May. Perhaps the plankton of the spring bloom subsist on stored P, acquired as orthophosphate very early in the year, but for most phytoplankton, intracellular P stores cannot explain the discrepancy between uptake and growth. I therefore reject orthophosphate uptake as having been the principal supplier of P for phytoplankton growth in Memphremagog.

Since this conclusion depends upon the accuracy of my estimates of P and C doubling times, it is reasonable to inquire how robust these estimates are. First, consider C. Several recent lines of evidence indicate that the C doubling measured by the ^{14}C technique should be reasonably close to net productivity (Jackson 1983; Smith et al. 1984; Platt et al. 1984) in oligotrophic systems. Even if the adequacy of the ^{14}C production technique were seriously in doubt, the P doubling rates measured here are not only very much smaller than my growth rate estimates, they are also much smaller than any other published in situ growth rates (see compilation by Goldman et al. 1979, table 2; also Lund 1964; Ahlgren 1970; Peterson et al. 1974; Knoechel and Kalff 1975; Smith and Kalff 1981). Thus, it is unlikely that the ^{14}C results could be sufficiently in error to change the interpretation of my results.

The P doubling data may admit a very different interpretation. The P doubling rate in this study is proportional to the orthophosphate concentration, as measured by Rigler's (1966) bioassay. Several other techniques to measure orthophosphate have been proposed (Martin and Doty 1949; Menzel and Corwin 1965; Shapiro et al. 1969; Peters 1977; Petterson 1979); most yield higher concentration estimates than does Rigler's technique. Exactly what each technique measures is still moot. Methods that employ the molybdenum-blue reaction (Martin and Doty 1949; Menzel and Corwin 1965; Shapiro et al. 1969) are not wholly specific to orthophosphate, and they can therefore grossly overestimate PO_4, especially in oligotrophic lakes (Rigler 1968; Chamberlain and Shapiro 1969; Stainton 1980; Tarapchak et al. 1982). They may nonetheless measure something close to "biologically available P" (Nürnberg and Peters 1984), but this is small consolation if one wishes to test hypotheses specifically about orthophosphate. Petterson's (1979) enzymatic technique looks promising, but it has not yet been extensively tested. Rigler's and Peters' bioassays have the advantage that they are based on the movements of the chemically defined [^{32}P]orthophosphate ion. They should therefore be very specific, involving no other chemical forms. After 20 yr of use by numerous investigators, there is no evidence of which I am aware to gainsay the Rigler orthophosphate estimates. D. R. S. Lean (National Water Research Institute, Burlington, Ont., pers. comm.) has suggested that the Rigler estimate may be influenced by the fact that different organisms are responsible for orthophosphate uptake at high and low concentrations (Lean and White 1983). This suggestion has not yet been tested, but it seems unlikely to be important in the present case, since the P additions were so small (0.82–6.45 nmol·L^{-1}). On the other hand, if I had used

Peters' (1977) measurements of SRP in Lake Memphremagog as representing orthophosphate, then I would have concluded that orthophosphate uptake supplied 2–3 times as much P as the algae required. I feel that the extant evidence still supports the bioassays as the best estimators of orthophosphate; however, one might also interpret my data as indicating that the Rigler technique and the many studies that have used it are seriously flawed.

The conclusion that phytoplankton obtained little P from orthophosphate appears otherwise to be reasonably conservative. First, as mentioned earlier, Rigler's bioassay for orthophosphate would cause the gross uptake of orthophosphate to be overestimated. Phytoplankton also excrete P, such that their gross uptake of P can exceed the amount retained for growth by more than an order of magnitude (Nalewajko and Lean 1978; Lean and White 1983). Under these conditions, orthophosphate uptake would supply as little as 0–1% of the total amount of P utilized for growth. On the other hand, all particulate P was assumed to be in living biomass. This is likely to result in some underestimation of P doubling rates. The underestimation is probably small, since detrital P should be leached and mineralized quite quickly (Garber 1984). My conclusions would also be weakened if the ^{14}C technique significantly overestimated daily net production, contrary to the contentions of Jackson (1983), Smith et al. (1984), and Platt et al. (1984). This might result from the fact that I made no explicit correction for night respiration. Carbon storage by P-limited algae (e.g. Healey and Hendzel 1975) would not affect my conclusions, provided that the quantity of stored C is not rapidly changing; my C doubling rates would therefore represent the time required to double both the essential and the surplus cell C.

An hypothesis has been proposed by Goldman et al. (1979) to explain an analogous discrepancy between apparently high algal growth rates and vanishing nutrient concentration in the sea. It postulates that phytoplankton may profit from microscale patches of elevated nutrient concentrations created by zooplankton excretion. Essentially, Goldman et al. (1979) argue that whenever a phytoplankton cell encounters a patch of concentrated nutrients, its higher uptake rate would permit the cell to satisfy its nutrient requirements very quickly. There is considerable doubt that such patches persist long enough to supply a significant amount of nutrient (Jackson 1980; Williams and Muir 1981; Currie 1984a). However, even if they did, the average nutrient uptake by phytoplankton would actually be diminished in a patchy environment, relative to a homogeneous environment, for reasons detailed earlier (Currie 1984b). The discrepancy between uptake and growth would therefore be exacerbated by the presence of nutrient patches.

It also might be suggested that bacteria rapidly recycle P whereas algae may have a superior ability to retain it. Algal uptake in this study was measured from the ambient orthophosphate concentration and the rate constant of orthophosphate uptake. These are both independent of how quickly P is recycled or how well it is retained. Differences in P recycling or storage cannot mitigate insufficient gross P uptake by algae.

One may inquire to what extent the P partitioning in Lake Memphremagog is typical of that in other lakes. I have no data that permit a direct test; however, the orthophosphate partitioning patterns observed here were very similar to those observed in many other P-limited lakes, where bacteria appear to dominate orthophosphate uptake (Rigler 1956; Burnison 1975; Koschel 1980; Lean and White 1983; Berman 1985; Currie et al. 1986). There may be exceptions to this generalization. Paerl and Lean (1976) and Levine et al. (1986) reported algal dominance of orthophosphate uptake in P-deficient lakes, although their experiments were not specifically designed to show this. A further exception may be Lake Michigan, where phytoplankton apparently do compete successfully for orthophosphate (S. Tarapchak and R. Moll, Great Lakes Environmental Research Laboratory, NOAA, Ann Arbor, MI, unpubl. data). At present, I cannot explain these discrepancies. It must also be noted that, in lakes where P is not strongly limiting, the algae apparently are responsible for a much larger portion of the total community orthophosphate uptake (Koschel 1980; Berman 1985; Currie et al. 1986). It is interesting that within a single lake (Kinneret; Berman 1985) the same pattern holds: when ^{32}P turnover is slow, algae dominate orthophosphate; when it is rapid, bacteria do (Currie et al. 1986). When P is abundant, the phytoplankton probably can derive sufficient P from orthophosphate uptake, but under most P-limiting conditions, they do not.

The conclusion that algae do not get their P from orthophosphate, at least for most of the summer, certainly begs the question of where they do get P. An obvious possibility is the uptake of dissolved organic P (DOP). This is a very difficult hypothesis to test directly, due to the numerous chemical forms that DOP may include in situ. There is at least some evidence that is consistent with this hypothesis (Aaronson and Patni 1976; Taft et al. 1977; Currie et al. 1986). A second possibility is that algae obtain P by some process other than direct uptake from solution — perhaps transfer between bacteria and algae (cf. Paerl and Lean 1976). For the moment, the question of where algae in oligotrophic systems do get their P remains open.

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Influence of Water Hardness, pH, and Alkalinity on the Mechanisms of Copper Toxicity in Juvenile Rainbow Trout, *Salmo gairdneri*

Darrel Jon Laurén and D. G. McDonald

Department of Biology, McMaster University, Hamilton, Ont. L8S 4K1

Laurén, D. J., and D. G. McDonald. 1986. Influence of water hardness, pH, and alkalinity on the mechanisms of copper toxicity in juvenile rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 43: 1488–1496.

Juvenile rainbow trout were exposed to 25–400 μg copper·L^{-1} for 24 h. Water hardness, pH, and alkalinity were varied independently at a constant [Na^+]. Net and unidirectional sodium fluxes were measured in hard and soft, low-alkalinity water and in hard, high-alkalinity water at neutral pH and pH 5.0. In low alkalinity water, Na^+ uptake (J_{in}) was inhibited at copper concentrations as low as 25 μg·L^{-1}, and sodium efflux (J_{out}) was stimulated above 100 μg·L^{-1}. High-alkalinity water significantly reduced the effects of copper on J_{in} and J_{out}, but there was no significant effect of increasing water hardness. The effects of pH 5.0 and copper were additive from 25 to 100 μg·L^{-1}, but a pure copper effect was found from 200 to 400 μg·L^{-1}. Fish died when they had lost about 50–55% of their exchangeable Na^+ pool. Water hardness and alkalinity had no effect on the apparent uptake of copper, but copper uptake was reduced by about 50% at pH 5.0.

De jeunes truites arc-en-ciel (*Salmo gairdneri*) ont été exposées à 25–400 μg·L^{-1} de cuivre pendant 24 h. La dureté de l'eau, le pH et l'alcalinité ont été modifiés indépendamment, le [Na^+] demeurant constant. Les flux nets et unidirectionnels de sodium ont été mesurés dans de l'eau dure et douce, à basse alcalinité, et dans de l'eau dure, à haute alcalinité, à un pH neutre et à un pH de 5,0. Dans l'eau de basse alcalinité, l'assimilation de Na^+ ($J_{entrée}$) a été paralysée à des concentrations de cuivre aussi basses que 25 μg·L^{-1}, et le dégagement de sodium (J_{sortie}) a été stimulé à des concentrations supérieures à 100 μg·L^{-1}. L'eau de haute alcalinité a réduit de façon significative les effets du cuivre sur $J_{entrée}$ et J_{sortie}, mais une augmentation de la dureté de l'eau n'a pas eu d'effet notable. Un pH de 5,0 et le cuivre ont eu un effet cumulatif entre 25 et 100 μg·L^{-1}, mais on a observé un effet cuivre pur entre 200 et 400 μg·L^{-1}. Les poissons sont morts après avoir perdu de 50 à 55 % de la quantité totale de leur Na^+ échangeable. La dureté et l'alcalinité de l'eau n'ont pas eu d'effet sur la captation apparente du cuivre, mais cette dernière a été réduite d'environ 50 % à un pH de 5,0.

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Copper is one of the more abundant transition metals and can be acutely toxic to freshwater fish in the 10–1000 μg·L^{-1} range (Spear and Pierce 1979). Toxic concentrations occur in nature mainly through such processes as mining and smelting, and through the leaching of copper from bedrock due to acid mine drainage and acid precipitation (Hodson et al. 1979; Spry et al. 1981). Furthermore, copper production has increased steadily during the twentieth century, and Nriagu (1979) has estimated that about 30% of the all time atmospheric emissions of copper will occur in the present decade, 1980–90. Thus, the assessment of copper toxicity has been the subject of numerous studies and reviews (Spear and Pierce 1979; Hodson et al. 1979).

The results of these studies have shown that increasing water hardness, pH, or alkalinity all may reduce the lethality of copper to fish (Shaw and Brown 1974; Zitko and Carson 1976; Howarth and Sprague 1978; Chakoumakos et al. 1979; Miller and Mackay 1980). Since alkalinity and pH control the equilibrium between toxic and nontoxic copper species (Stiff 1971; Howarth and Sprague 1978; Chakoumakos et al. 1979), some of these effects may be understood in physico-chemical terms. On the other hand, water hardness (i.e. calcium) does not affect copper speciation, but may be understood in physiological terms. For example, several studies have shown that calcium affects gill permeability in fishes (Potts and Fleming 1971; Cuthbert and Maetz 1972), and Pagenkopf (1983) has suggested that calcium may also compete with metals for binding sites on the gills. Thus, physiological studies of copper toxicity to fish may reveal mechanisms of action which cannot be addressed in lethality studies alone.

In a previous physiological study of the short-term effects of copper on adult rainbow trout, *Salmo gairdneri*, we showed that toxicity results from the disruption of gill ionoregulatory function at concentrations as low as 12.5 μg·L^{-1} (Laurén and McDonald 1985). We further showed that this occurred by both the inhibition of sodium influx and the stimulation of sodium efflux. However, our study was conducted in low-alkalinity water only, and we were unable to find a significant effect of either water hardness (within the range of 25–1000 μ*M*) or pH (within the range of pH 7.8–5.0) on net sodium balance within 24 h. Since all of the lethality studies cited above were conducted on juvenile trout, we wondered if these differences in the response of trout to the modulatory effects of water hardness and pH on copper toxicity could be explained by the

TABLE 1. Ionic composition (μM) of test media at the beginning of flux periods for all experiments. Values are means ± 1 SEM. HL = high Ca^{2+}, low alkalinity; SL = low Ca^{2+}, low alkalinity; HH = high Ca^{2+}, high alkalinity.

Treatment	pH	$[Na^+]$	$[Cl^-]$	$[Ca^{2+}]$	$[K^+]$	[Ammonia]
HL	7.8	237±6	192±5	1064±20	6.4±1.1	8.6±0.5
SL	7.8	223±2	187±1	27±4	3.9±0.4	10.6±0.4
HL	5.0	257±3	234±4	1047±11	15.3±0.6	126±1.5
SL	5.0	241±3	238±7	31±6	11.1±0.6	110±3.8
HH	8.1	219±3	207±14	1103±28	—	12.7±1.6

greater sensitivity of smaller fish. Furthermore, we have not previously examined the effects of high alkalinity on the copper-dependent disruption of branchial ionoregulatory function. Therefore, the objectives of this study were to (1) examine the relative roles of hardness, alkalinity, and pH in modulating the toxicity of copper in juvenile trout and (2) compare these results with those found with adults exposed to copper under similar conditions. Unidirectional and net sodium fluxes were used again as a sensitive indicator of copper toxicity.

Methods and Materials

Animals

Juvenile rainbow trout, obtained from Spring Valley Trout Farm, Petersburg, Ontario, were maintained in flowing, tap-dechlorinated water. The fish were fed every other day, and mortalities were less than 1% per week. The $[Na^+]$ and $[Ca^{2+}]$ of the holding system were about 600 and 1000 μM, respectively, and temperature was maintained at 15 ± 2°C. The mean wet weight of the fish used in these experiments was 2.46 ± 0.02 g (n = 495).

Exposure Media

All flux media were made from distilled water with salts added. To facilitate net Na^+ measurements, and to more closely represent the $[Na^+]$ of those waters which are most likely to suffer from elevated copper concentrations due to acid precipitation, fish were acclimated for 14 d to about 200 μM Na^+ (Table 1). For those experiments conducted in hardwater of low alkalinity (HL), the $[Ca^{2+}]$ of the acclimation medium was about 1000 μM, and for softwater, low-alkalinity experiments (SL), the $[Ca^{2+}]$ was about 25 μM (Table 1). Calcium was added as $Ca(NO_3)_2$. For experiments conducted at pH 5.0, 0.1 M HNO_3 was used to titrate the water, and the pH was monitored and adjusted every 15–30 min. Artificial hard, high-alkalinity water (HH) was prepared by adding 1000 μM $CaCO_3$ and 200 μM Na^+ to distilled water. The $CaCO_3$ was dissolved by bubbling with CO_2, and the pCO_2 was reduced to normal levels by bubbling with humidified air for 24 h. Fish were acclimated for 14 d to about 200 μM Na^+ and 1000 μM $Ca(NO_3)_2$ as in the other experiments using artificial hard-water. Alkalinity was measured by titrating a known volume to pH 4.0 with 0.02 M HCl. All experiments were conducted at 15 ± 1°C.

Ion Flux Measurements

Fish were exposed to copper in 2-L square bags constructed from polyethylene sheeting and heat sealed along the seams with a commercial bag sealer. Polyethylene was chosen because copper uptake onto the plastic was less than 5% over a 24-h period at neutral pH, and even lower at pH 5.0. Groups of five fish were exposed for 24 h to 25, 50, 100, 200, or 400 μg copper·L^{-1} in 1 L of the appropriate medium (Table 1), so as to form a matrix of high and low hardness, alkalinity, and pH. To reduce the stress of confinement, the fish were first transferred to an identical flux bag for 12 h. The flux measurements were started by transferring the fish to bags containing the appropriate copper concentration and ^{22}Na at 37 kBq·L^{-1}. Water samples were collected at 4-h intervals. After 24 h, or at the time of death, whole fish were counted in a Nuclear Chicago gamma counter and sodium uptake (J_{in}) determined calculated as

$$(1)\quad J_{in} = \frac{(([Na^+]_{i\to t}/Q_{i\to t})Q_f)}{g\cdot t}$$

where $[Na^+]_{i\to t}$ is the mean sodium concentration of the exposure media from the beginning of the flux until 24 h, or death of the fish, $Q_{i\to t}$ is the mean counts per minute of the exposure media from the beginning of the flux period until 24 h, or the death of the fish, Q_f is the counts per minute in the whole fish, g is the weight to the nearest 0.01 g, and t is the time of death in hours.

Whole body copper uptake was measured simultaneously with ^{22}Na uptake, using ^{64}Cu (3.1 MBq·L^{-1}) and counting whole fish. For this purpose we used the same formula as for J_{in} (after first correcting for the decay of ^{64}Cu, $t_{1/2}$ = 768 min), but did not divide by t.

After allowing sufficient time for the complete decay of ^{64}Cu, and recounting of the fish for ^{22}Na uptake alone, each fish was digested in 5 mL of concentrated nitric acid and whole body total $[Na^+]$ measured by atomic absorption. From this information, it was possible to estimate net losses of whole body $[Na^+]$ in individual fish as

$$(2)\quad J_{net} = \frac{[Na^+]_C - [Na^+]_T}{g\cdot t}$$

where $[Na^+]_C$ is the average whole body $[Na^+]$ for 20 control fish, $[Na^+]_T$ is the whole body total $[Na^+]$ for the particular treatment in micromoles per gram wet weight, and g and t are wet weight and time to death, respectively.

Sodium efflux (J_{out}) was calculated as

$$(3)\quad J_{out} = J_{in} - J_{net}.$$

To assess the allometric differences in the effect of copper on juvenile and adult trout, it was necessary to compare changes in whole body exchangeable sodium. Whole body exchangeable $[Na^+]$ of control fish ($[Na]_{exC}$) was determined as

$$(4)\quad [Na]_{exC} = {}^{22}Na\ space\cdot[Na^+]_{plasma}.$$

Blood was collected by caudal transection, and $[Na^+]_{plasma}$ was measured by atomic absorption. Mayer and Nibelle (1969)

showed that the sodium space of eels is uniformly labelled after about 10 h of exposure in ^{22}Na-labelled water. To assure uniform labelling of the sodium space in trout, we exposed juvenile rainbow trout (n = 33) for 24 h in HL labelled with ^{22}Na at 148 kBq·L^{-1} and calculated the ^{22}Na space as

(5) ^{22}Na space

$$= (\text{cpm} \cdot \text{g wet weight}^{-1})/(\text{cpm} \cdot \text{mL plasma}^{-1})$$

Whole fish were counted for the determination of cpm per gram wet weight, and 5-μL aliquots of plasma were collected after caudal transection and counted for the determination of cpm per millilitre plasma. $[Na^+]_{plasma}$ and cpm per millilitre plasma were measured on the same samples.

Because whole body chloride cannot be measured in acid digests, and whole body potassium was variable, fluxes of chloride and potassium were calculated from changes in their concentrations in the media. Ammonia fluxes were also calculated from changes in the concentration of ammonia in the media. Sodium and potassium were measured with a Varian 1275 atomic absorption spectrophotometer, chloride was measured with a Cotlove chloride titrator, and ammonia was measured with a micro-modification of the method of Verdouw et al. (1978).

Estimation of 24-h LC50

Mortalities were recorded at hourly intervals at all copper concentrations, and the 24-h LC50 was estimated by the method of Litchfield and Wilcoxon (1949).

Copper Uptake versus Sodium Uptake

In a separate experiment designed to examine the correlation between the accumulation of copper and the inhibition of the sodium uptake rate, fish were exposed to 50 μg copper·L^{-1} in HL labelled with ^{64}Cu (1.5 MBq·L^{-1}) and ^{22}Na (37 kBq·L^{-1}). Groups of five fish were removed at regular intervals for up to 24 h. Whole fish were counted and sodium and copper uptake calculated as described by equation 1.

Expression of Results

Mean values ± 1 standard error of the mean (SEM) are given. Student's t-test was used in the statistical analysis of the results.

Results

Control Fish

No mortalities were found among control animals at neutral pH in HL, SL, or HH. Whole body $[Na^+]$ was 54.8 ± 3.0 and 54.4 ± 2.1 μmol·g^{-1} (n = 20) in HL and SL fish, respectively. Whole body $[Na^+]$ for HH fish was 65.0 ± 1.4 μmol·g^{-1} (n = 20). This change occurred over the 4-mo period between the two groups of experiments. Radiosodium space was determined to be 27 ± 4% (n = 33) and plasma $[Na^+]$ 152 ± 5 mM (n = 33); therefore, the exchangeable Na^+ pool at neutral pH was determined to be 41.6 μmol·g^{-1}, or about 75% of the total whole body $[Na^+]$. For control fish, net sodium fluxes could not be reliably calculated from changes in whole body $[Na^+]$ because the changes were so small. Therefore, they were calculated from changes in the $[Na^+]$ of the flux media. Although control fish initially lost ions immediately after being transferred from the acclimation bath to the flux bath, net Na^+ fluxes at neutral pH were +51 ± 30, +35 ± 53, and +25 ± 20 nmol·g^{-1}·h^{-1} for HL, SL, and HH fish, respectively (Fig. 1a). Control fish also lost K^+ and Cl^- immediately after transfer, but again, these losses were negligible by the end of the 24-h flux period (Table 2). Ammonia efflux was about −1000 nmol·g^{-1}·h^{-1} in HL, SL, and HH (Table 2). Sodium uptake (J_{in}) was about 600 nmol·g^{-1}·h^{-1}, and there was no significant difference between HL, SL, and HH (Fig. 1b). Because the fish were in sodium balance for the 24-h flux period, sodium efflux was also about 600 nmol·g^{-1}·h^{-1} in all control conditions (Fig. 1c).

Effects of Exposure to pH 5.0

Control fish exposed to pH 5.0 without copper lost between 7 and 10% of their exchangeable Na^+ within 24 h, but no mortalities occurred (Table 3). Net K^+ losses were also found, but these were not significantly different from those at neutral pH (Table 2). Net chloride losses were found, but were always lower than Na^+ losses (Table 2). There was no significant effect of pH on ammonia efflux (Table 2). Sodium uptake was inhibited by about 25% in HL and by about 50% in SL at pH 5.0 (Fig. 1b). This was the only significant effect of water hardness. Sodium efflux (J_{out}) was about the same in HL at pH 5.0 as at neutral pH, but was about 28% lower in SL at pH 5.0 than at neutral pH (Fig. 1c).

Effects of Copper Exposure on Net Ion Balance

At neutral pH, copper exposure led to concentration-dependent net ion losses (Fig. 1a; Table 2). Exposure to as little as 25 μg copper·L^{-1} led to net Na^+, K^+, and Cl^- losses within 24 h (Fig. 1a; Table 2), but there was no significant effect of water hardness within this time. A significant inhibition of ammonia efflux was found at 400 μg copper·L^{-1} at neutral pH in both HL and SL (Table 2).

At pH 5.0, exposure to copper either led to greater ion losses or had no effect on ion losses, depending upon the copper concentration. Fish exposed to 25 to 50 μg copper·L^{-1} at pH 5.0 experienced larger net Na^+ losses than those exposed at neutral pH, but there was no significant effect of either pH or hardness from 100 to 400 μg·L^{-1} (Fig. 1a). There was no significant effect of pH on either K^+ or Cl^- net losses at any copper concentration (Table 2). Ammonia efflux was significantly inhibited at 200 and 400 μg copper·L^{-1} in SL, but not in HL at pH 5.0 (Table 2).

In HH, copper exposure led to only about one third of the ion losses found in low alkalinity water (Fig. 1a; Table 2). Exposure of fish to copper in HH significantly reduced the effect of copper on net Na^+ and Cl^- losses, but had no effect on net K^+ losses (Fig. 1a; Table 2). No net Na^+ or Cl^- losses were found at 25 μg·L^{-1}. Ion losses increased as copper concentration increased from 50 to 400 μg·L^{-1}, but the ameliorative effect of HH was also more apparent as the copper concentration increased (Fig. 1a; Table 2). Alkalinity had no significant effect on ammonia efflux (Table 2).

Effects of Copper on Sodium Influx

Exposure of juvenile trout to copper resulted in a concentration-dependent inhibition of sodium influx (J_{in}). At neutral pH the inhibition of J_{in} appeared to be logarithmic with copper concentration, with most of the inhibition of J_{in} occurring

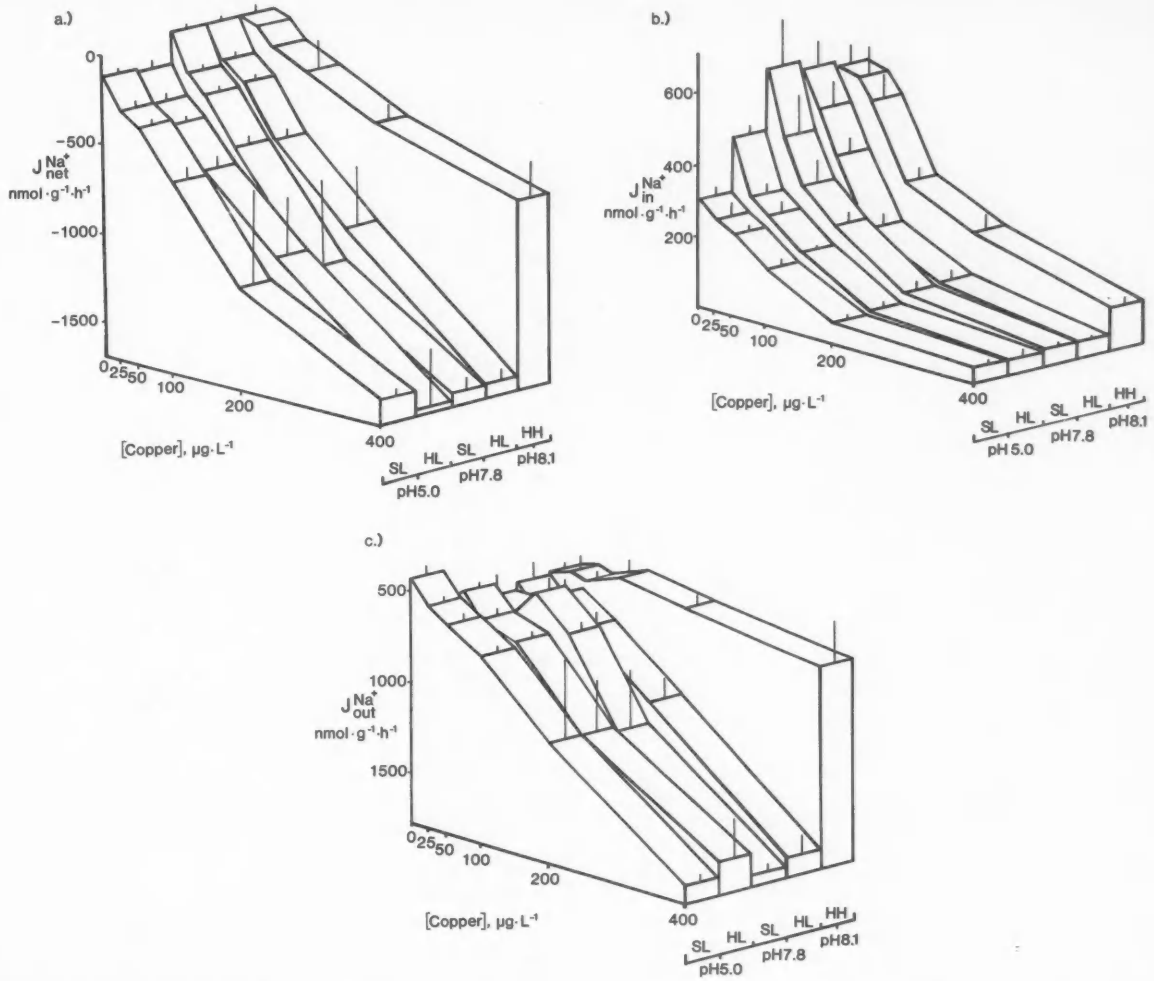

FIG. 1. Sodium balance. (a) Net sodium loss rate (mean ± SEM) of juvenile rainbow trout exposed to copper for 24 h, or until death. For high- (HL) and low- (SL) Ca^{2+}, low-alkalinity water at neutral pH, $n = 15$; for high- (HL) and low- (SL) Ca^{2+}, low-alkalinity water at pH 5.0, $n = 10$; and for high- (HH) Ca^{2+}, high-alkalinity water at neutral pH, $n = 20$. (b) Sodium influx rate. Sample size as in Fig. 1a, except that $n = 10$ in HH. (c) Sodium efflux rate. Sample size as in Fig. 1b.

between 25 and 100 $\mu g \cdot L^{-1}$ (Fig. 1b). At 100 $\mu g \cdot L^{-1}$ J_{in} was inhibited by about 65% (Fig. 1b). There was no significant effect of water hardness, except in control fish at pH 5.0 (Fig. 1b). Nevertheless, from 25 to about 100 $\mu g \cdot L^{-1}$, the combination of exposure to pH 5.0 and copper led to a greater inhibition of J_{in} than was found for copper exposure alone (Fig. 1b), and at 100 $\mu g \cdot L^{-1}$, J_{in} was inhibited by about 72% (Fig. 1b). There was no effect of pH on J_{in} at 200 or 400 μg copper $\cdot L^{-1}$ (Fig. 1b), and the effect of pH was not strictly additive with that of copper even at the lower copper concentrations (Fig. 1b).

The inhibitory effect of copper on J_{in} was significantly reduced by exposure in HH (Fig. 1b). There was no significant effect of copper at 25 $\mu g \cdot L^{-1}$, and copper was about 20–30% less inhibitory at all copper concentrations tested (Fig. 1b). As in HL and SL at neutral pH, most of the inhibition of J_{in} occurred between 25 and 100 μg copper $\cdot L^{-1}$ (Fig. 1b).

Effects of Copper on Sodium Efflux

Sodium efflux (J_{out}) was less sensitive to copper than J_{in}. There was no significant effect of copper on J_{out} from 25 to 50 μg copper $\cdot L^{-1}$ in HL or SL at either neutral pH or pH 5.0 (Fig. 1c). However, a significant increase in J_{out} was found from 100 to 200 $\mu g \cdot L^{-1}$ in HL and SL at both neutral pH and pH 5.0 (Fig. 1c).

In contrast with HL and SL, no stimulation of J_{out} was found in HH until between 200 and 400 μg copper $\cdot L^{-1}$ (Fig. 1c).

Mortalities

Fish died when they had lost about 55% of their whole body exchangeable Na^+. This occurred at 200 and 400 μg copper $\cdot L^{-1}$ in HL and SL fish at neutral pH and pH 5.0, but only at 400 $\mu g \cdot L^{-1}$ in HH (Table 3). There was no significant effect of either water hardness or pH on mortality during the

TABLE 2. Net Cl^-, K^+, and ammonia fluxes ($nmol \cdot g^{-1} \cdot h^{-1}$) in juvenile rainbow trout exposed to copper for 24 h. For neutral pH (HL and SL), $n = 15$, for pH 5.0 (HL and SL), $n = 10$, and for HH, $n = 20$. Values are means ±1 SEM. Asterisks denote significant differences from control values ($P < 0.05$). HL = high Ca^{2+}, low alkalinity; SL = low Ca^{2+}, low alkalinity; HH = high Ca^{2+}, high alkalinity.

		Copper ($\mu g \cdot L^{-1}$)					
Fluxes	pH	0	25	50	100	200	400
J_{net} Cl^-							
HL	7.8	+70±180	−304±82	−346±119	−632±102*	−1087±522*	−1632±404*
SL	7.8	+110±77	−282±180	−363±67*	−527±120*	−1340±126*	−2211±787*
HL	5.0	−138±108	−338±116	−394±177	−621±147*	−1002±55*	−1024±153*
SL	5.0	−60±53	−301±106*	−365±77*	−499±87*	−1068±208*	−1132±141*
HH	8.1	+66±44	−11±34	−112±17	−225±54*	−420±102*	−740±176*
J_{net} K^+							
HL	7.8	−54±11	−92±5	−122±12*	−137±36*	−253±32*	−352±48*
SL	7.8	−62±9	−112±25	−125±18*	−136±28*	−260±40*	−328±64*
HL	5.0	−110±55	−112±16	−133±28*	−139±33*	−195±50*	−223±39*
SL	5.0	−64±8	−109±18	−130±25*	−142±28*	−185±51*	−234±18*
HH	8.1	−48±15	−86±22	−117±10*	−136±10*	−200±38*	−244±33*
J_{net} Ammonia							
HL	7.8	−1016±181	−1175±188	−959±168	−880±178	−897±228	−637±186*
SL	7.8	−947±56	−876±107	−907±107	−1213±288	−566±42*	−543±207*
HL	5.0	−907±52	−826±46	−961±52	−931±64	−1160±194	−1013±173
SL	5.0	−1003±52	−1041±81	−869±123	−893±62	−893±80	−629±96*
HH	8.1	−1010±59	−987±23	−1021±143	−976±58	−963±102	−852±97

TABLE 3. Change in mean percent whole body exchangeable sodium in juvenile and adult rainbow trout exposed to copper for 24 h. Adult data from Laurén and McDonald (1985). HL = high Ca^{2+}, low alkalinity; SL = low Ca^{2+}, low alkalinity; HH = high Ca^{2+}, high alkalinity.

			Copper ($\mu g \cdot L^{-1}$)				
Treatment	pH	Control	25	50	100	200	400
HL	7.8						
Juveniles		+0.3	−10.8	−14.5	−32.3	−54.2	−65.6
Adults		−0.4	−6.1	−9.2	−15.3	−21.6	ND
SL	7.8						
Juveniles		−0.5	−11.5	−16.2	−30.6	−63.9	−60.8
Adults		+2.8	−7.2	−8.6	−12.8	−24.3	ND
HL	5.0						
Juveniles		−10.1	−17.9	−23.2	−36.2	−56.9	−53.2
Adults		−9.2	ND	ND	−14.8	−21.7	ND
SL	5.0						
Juveniles		−7.4	−17.1	−20.1	−34.7	−65.6	−60.8
Adults		−4.7	ND	−13.6	−20.5	ND	ND
HH	8.1						
Juveniles		−0.4	−0.5	−1.0	−2.7	−11.5	−39.5
Adults		ND	ND	ND	ND	ND	ND

ND = not determined.

24 h of copper exposure, and the predicted 24-h LC50 for both HL and SL fish at neutral pH and pH 5.0 was about 90 $\mu g \cdot L^{-1}$ (Fig. 2). The slopes of the time to death versus copper concentration for HL and SL at neutral pH and pH 5.0 were parallel, indicating no differences in the mechanism of toxicity (Fig. 2). Insufficient mortalities occurred in HH for the accurate estimation of a 24-h LC50, but the few mortalities which did occur suggest that this concentraiton is in excess of 400 μg copper $\cdot L^{-1}$.

Copper Uptake

Copper uptake among fish which survived for 24 h was linear with copper concentration up to about 100 $\mu g \cdot L^{-1}$, and then leveled off (Fig. 3). There was no significant effect of water hardness on copper uptake by juvenile trout at neutral pH (Fig. 3). However, copper uptake was significantly higher among fish which died before 24 h. At 200 and 400 $\mu g \cdot L^{-1}$, copper uptake in fish which died was 441 ± 69 and 1210 ± 111 $ng \cdot g^{-1}$ in HL and 594 ± 71 and 1359 ± 186 $ng \cdot g^{-1}$ in SL, whereas copper uptake in survivors reached a maximum of about 360 ± 50 $ng \cdot g^{-1}$ (Fig. 3).

There was a significant inhibition of copper uptake at pH 5.0 (Fig. 3). Again, there was no significant effect of water hardness, and copper uptake leveled off at about 100 $\mu g \cdot L^{-1}$

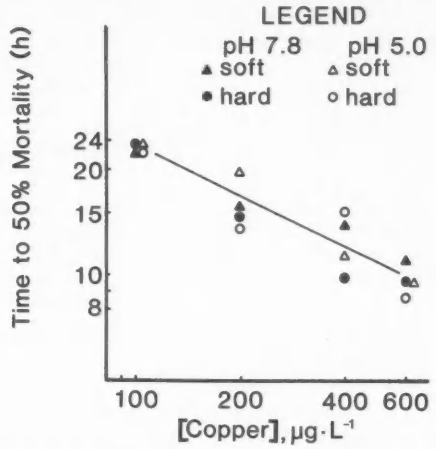

FIG. 2. Toxicity curve for copper-exposed juvenile rainbow trout. For neutral pH, high- and low-Ca^{2+}, low-alkalinity water, $n = 15$; for pH 5.0, high- and low-Ca^{2+} water, $n = 10$. Line fitted by eye.

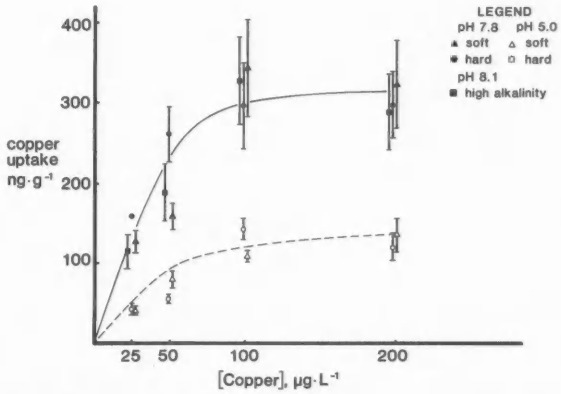

FIG. 3. Copper uptake (mean ± SEM) of juvenile rainbow trout which survived exposure to copper for 24 h. For neutral pH, high- and low-Ca^{2+}, low-alkalinity water, $n = 15$; for pH 5.0, high-Ca^{2+} water, $n = 10$; and for high-Ca^{2+}, high-alkalinity water at neutral pH, $n = 10$.

(Fig. 3). Copper uptake was also significantly greater among fish which died before 24 h than those which survived for 24 h. At 200 and 400 $\mu g \cdot L^{-1}$, the copper uptake for fish which died was 534 ± 86 and 816 ± 90 $ng \cdot g^{-1}$ in HL and 497 ± 63 and 1221 ± 166 $ng \cdot g^{-1}$ in SL at pH 5.0. Thus, there was less effect of pH on copper uptake among fish which died before 24 h than among survivors (Fig. 3).

HH did not significantly affect copper uptake relative to HL or SL (Fig. 3).

Copper Uptake versus Sodium Uptake

This experiment revealed that at least at 50 μg copper $\cdot L^{-1}$ in HL at neutral pH, copper uptake was not constant with time, and the rate of sodium uptake was progressively reduced (Fig. 4a). The copper uptake rate during the first hour was 88.42 ± 11.69 $ng \cdot g^{-1} \cdot h^{-1}$; this accounted for nearly 50% of the total copper accumulated during 24 h (Fig. 4a). The time-

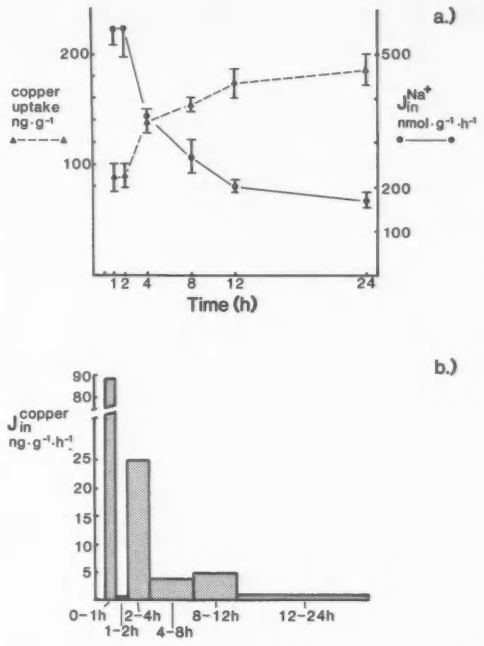

FIG. 4. (a) Copper uptake (▲) and sodium uptake rate (●) of juvenile rainbow trout exposed to 50 μg copper $\cdot L^{-1}$ in low-alkalinity hard-water at neutral pH, for varying periods of time (means ± 1 SEM, $n = 5$). (b) Copper uptake rate (means, $n = 5$). These values were calculated by subtracting the copper uptake during the previous exposure period from the copper uptake from time 0 to time t and dividing the difference by t.

weighted mean copper uptake rate from 2 to 24 h was only 4.17 ± 1.42 $ng \cdot g^{-1} \cdot h^{-1}$ (Fig. 4b). No inhibition of J_{in} was found during the first 2 h of copper exposure (Fig. 4a). From 2 to 4 h, the copper uptake rate had increased above the 2-h value (Fig. 4b), and J_{in} was significantly inhibited (Fig. 4a). Copper continued to accumulate at a slower rate, and J_{in} continued to decrease for the remainder of the exposure (Fig. 4a).

Discussion

Effects of Alkalinity on Copper Toxicity

By varying only alkalinity while maintaining hardness, $[Na^+]$, and pH constant, we have shown that alkalinity is a major modulator of short-term copper toxicity to rainbow trout. This confirms the hypothesis of Stiff (1971) that differences in copper toxicity between hard and softwaters are dependent upon carbonate complexation capacity rather than hardness, per se. Similar conclusions were reached by Andrew (1976) for fathead minnows (*Pimephales promelas*), by Shaw and Brown (1974) for rainbow trout, and by Zitko and Carson (1976) for Atlantic salmon (*Salmo salar*).

Previous studies have shown that increases in alkalinity reduce copper toxicity to trout, but these studies used lethality (i.e. 96-h LC50) as the only indicator of the effects of water chemistry on copper toxicity (Shaw and Brown 1974; Zitko and Carson 1976; Howarth and Sprague 1978; Chakoumakos et al. 1979; Miller and Mackay 1980). In the present study, we have shown that the physiological basis for these ameliorative

effects of alkalinity lies in the reduction of the effects of copper on branchial ionoregulatory function. We have also shown that this protective effect is fast-acting (i.e. found within 24 h), and apparent at sublethal levels of copper.

Alkalinity affects the physical form of the toxicant (Stiff 1971) and thus may be properly considered as an external modulator of toxicity. This appears to be accomplished by way of the equilibrium between the toxic species, Cu^{2+}, $CuOH^{+}$, and $CuOH_2^0$, and the nontoxic carbonate species, $CuHCO_3^{+}$, $CuCO_3^0$, and $Cu(CO_3)_2^{2-}$ (Chakoumakos et al. 1979). Thus, in natural low-alkalinity waters, regardless of pH, noncarbonate species of copper would predominate (e.g. from about 99% at pH 5.0 to about 65% at pH 7.6). In the present study, carbonate species were kept low by the use of only calcium nitrate salts as opposed to calcium carbonate salts. We chose nitrate salts because nitrates are nontoxic in themselves and because copper nitrate dissociates completely in water (Sillén and Martell 1964). In contrast, in our high-alkalinity studies, nontoxic copper carbonate species would predominate as they would in natural high-alkalinity waters (e.g. about 67% at pH 7.4) (Chakoumakos et al. 1979; Howarth and Sprague 1978).

Effects of Hardness on Copper Toxicity

We have also confirmed that water $[Ca^{2+}]$, within the range of 25−1000 μM, plays no significant role in short-term copper toxicity (Laurén and McDonald 1985). Zitko and Carson (1976) also found no effect of water hardness on LT50 toxicity tests of Atlantic salmon exposed to copper. Nevertheless, Chakoumakos et al. (1979) and Miller and Mackay (1980) have shown a significant effect of water hardness on the 96-h LC50 and 15-d ILC50, respectively. These differences in the effect of calcium may be resolved in at least two ways. First, the fish used by Chakoumakos et al. (1979) and Miller and Mackay (1980) were acclimated to softwater for only 2−3 d. Such short acclimation times would increase the apparent effect of calcium by decreasing the fitness of the softwater fish (i.e. incomplete acclimation; see Mechanisms of Toxicity section). Second, Lloyd (1965) suggested that hardwater fish must lose calcium before they become as sensitive to toxic metals as softwater fish. At low pH, McDonald (1983) suggested that H^+ displaces calcium from the paracellular tight junctions of the branchial epithelium, leading to the stimulation of ion loss. However, no effect of hardness on low pH stressed rainbow trout was found until after 40 h of exposure to pH 4.3 (McDonald et al. 1980). Thus, even in fully acclimated softwater fish, the displacement of calcium takes longer than 24 h to be expressed in a measurable physiological response.

Calcium is known to exert considerable control over the permeability of the gill itself (Potts and Fleming 1971; Cuthbert and Maetz 1972; Oduleye 1975; Eddy 1975) and thus may be considered an internal modulator of toxicity. Removal of calcium from the medium stimulates ion loss and water uptake and initiates a suite of physiological responses, which over the course of several weeks leads to an increase in the thickness of the mucus coat (Wendelaar Bonga 1978) and increased levels of the calcium-binding protein calmodulin (Flik et al. 1984). The objective of these adjustments seems to be the maintenance of a high-calcium microenvironment around the branchial epithelium, a conclusion supported by the observations of McWilliams (1983) of higher calcium binding on the gills of softwater acclimated fish. Thus, sublethal or chronically toxic levels of copper may only slowly displace calcium from the gills, and this slow displacement may allow additional adaptive responses such as the proliferation of chloride cells (Laurén and McDonald 1985; Laurent et al. 1985) and the stimulation of calcium uptake (Perry and Wood 1985), which further act to retain calcium. Nevertheless, beyond about 40 h, softwater acclimated fish are still less resistant to low pH than hardwater fish (McDonald et al. 1980), and the same would be expected for fish exposed to copper.

Thus, it seems likely that alkalinity, as the most important determinant of copper speciation, is the major short-term determinant of copper toxicity, but that mucus- or membrane-bound calcium, as the major determinant of gill permeability, plays a significant longer-term role in modulating the biological response to this toxicant.

Effects of pH on Copper Toxicity

We have also confirmed our previous finding that exposure to copper at pH 5.0 does not significantly increase the toxicity of copper concentrations above 100 $\mu g \cdot L^{-1}$, but that the net Na^+ losses found from 0 to 100 $\mu g \cdot L^{-1}$ at pH 5.0 were the result of the independent effects of copper and H^+ (Laurén and McDonald 1985). This phenomenon may be explained by the ability of copper to compete directly with H^+ to inhibit sodium uptake, as well as the ability of high copper concentrations to stimulate sodium efflux (Laurén and McDonald 1985). Therefore, at high copper concentrations a pure copper effect is found. Since ionic copper, Cu^{2+}, is much more abundant at pH 5.0 than at neutral pH, and since $Cu(OH)_2$ is the major noncarbonate species expected at neutral pH in our system, the failure to find a difference in toxicity between neutral pH and pH 5.0 suggests that these are both toxic forms of copper.

We have also shown that H^+ alone causes significantly greater inhibition of J_{in} in SL than in HL juvenile trout (Fig. 1b, SL vs. HL). This effect of water hardness is the same as reported by McDonald et al. (1983) for adult trout exposed to pH 4.3, but is the opposite of what was found with adult trout at pH 5.0 (Laurén and McDonald 1985). This is probably due to the greater sensitivity of the juvenile fish used in the present experiments and the large difference in $[H^+]$ between pH 5.0 and pH 4.3.

Mechanisms of Toxicity

The mechanism of toxicity changed as the copper concentration increased: (1) between 25 and 100 $\mu g \cdot L^{-1}$, the inhibition of J_{in} was the major effect and (2) between 100 and 400 $\mu g \cdot L^{-1}$, the stimulation of J_{out} made a major contribution to toxicity. The effect of copper on J_{out} may be due to the displacement of calcium from the paracellular tight junctions, whereas the effects of copper on J_{in} are most likely due to the high affinity of copper for —SH groups on transport enzymes such as Na^+-K^+-ATPase (Stagg and Shuttleworth 1982). Thus, the mechanism of copper toxicity appears to be complex, with at least two major sites of biochemical and physiological action.

Juvenile rainbow trout die when they lose about 50−55% of their whole body exchangeable Na^+. In juveniles, this occurred at about 200 μg copper $\cdot L^{-1}$, a concentration which, in adults, resulted in only about a 23% reduction in exchangeable Na^+ (Table 3). Thus, copper causes about twice the net ion loss in juveniles as in adults. This is due largely to the twofold higher sodium efflux rate in juveniles, relative to adults, which may, in turn, be explained by the nearly twofold increase in the

weight-specific surface area of the gill (square centimetres per gram) in juveniles compared with adults (Morgan 1971).

We calculated a 24-h LC50 for juvenile rainbow trout of 90 $\mu g \cdot L^{-1}$ in low-alkalinity water, regardless of the hardness and pH. As the slopes of the mortality lines were not significantly different, there is no suggestion that the mechanism of toxicity changes with either hardness or pH. Howarth (1976) also found 24-h LC50 values of 75–125 $\mu g \cdot L^{-1}$ in low-alkalinity water. In our high-alkalinity experiments, too few fish died to enable the accurate estimation of the 24-h LC50. However, the few deaths that did occur suggest that this figure would be in excess of 400 $\mu g \cdot L^{-1}$. Thus, the effects of high alkalinity on sodium balance are well correlated with the amelioration of lethality.

Several authors have suggested that organic complexation, like carbonate complexation, may explain the wide range of 96-h LC50's found in different studies of copper toxicity (Spear and Pierce 1979; Alabaster and Lloyd 1980). By measuring whole body ions, we have identified yet another possible source for these discrepancies. When trout are transferred to media of a low $[Na^+]$ or $[Ca^{2+}]$, they lose Na^+ until they reduce J_{out} Na^+ and increase J_{in} Na^+. These losses are somewhat additive, such that fish exposed to both a reduced $[Na^+]$ and $[Ca^{2+}]$ lose more Na^+ than those exposed to only a reduced $[Na^+]$ or a reduced $[Ca^{2+}]$. Thus, unless the fish are first acclimated to their respective assay media for at least 2 wk prior to the beginning of an experiment, the fish would likely exhibit lower whole body $[Na^+]$ before the addition of toxicant (D. J. Laurén and D. G. McDonald, unpubl. data). Published acclimation times for 96-h LC50 determinations range from 2 to 3 d (Chakoumakos et al. 1979; Miller and Mackay 1980) to 13 d (Howarth and Sprague 1978). Furthermore, none of the previous studies (Howarth and Sprague 1978; Chakoumakos et al. 1979; Miller and Mackay 1980) varied hardness, alkalinity, and $[Na^+]$ independently. When alkalinity is adjusted with $NaHCO_3$ (Chakoumakos et al. 1979; Miller and Mackay 1980), or by diluting natural hardwater with distilled water (Shaw and Brown 1974; Howarth and Sprague 1978), up to 40-fold changes (i.e. 25–1000 μM as $CaCO_3$) in $[Na^+]$ occur. In this respect, it is interesting to note that Brown (1981) found that the addition of NaCl increased the survival of trout exposed to low pH.

Copper Uptake versus Toxicity

In 24-h experiments, apparent copper uptake was not well correlated with the physiological effects of copper. In high-alkalinity water, copper uptake was the same as in low-alkalinity water, but toxicity was reduced, while at pH 5.0, copper uptake was only about 50% that found at neutral pH, but the toxicity was the same. One possible reason for these discrepancies is that a large portion of the calculated whole body copper uptake may not have entered the body at all, but was apparently due to the binding of copper to the body surface alone, and that this high degree of surface binding may have masked small, but significant, differences in the amount of copper which actually penetrated the gill. At 50 μg copper $\cdot L^{-1}$ in HL at neutral pH, copper uptake was very high for the first hour of exposure, but decreased rapidly with time. Such a change in the rate of copper uptake may be interpreted as the rapid loading of a small volume pool, such as the body surface, followed by a much slower rate of transfer to the internal body tissues. Copper uptake during this period of rapid uptake accounted for about 50% of the total copper accumulated during 24 h.

The slower phase of copper uptake is apparently the only component of the total, short-term uptake which is important to toxicity, since the sodium uptake rate was progressively inhibited during the slower period of copper accumulation, 2–24 h, but no inhibition of the sodium uptake rate was found during the period of highest copper uptake rate. Despite the fact that the copper accumulated during this slower phase of copper uptake accounted for about 50% of the total copper accumulated during 24 h, we have found that more than 98% of the copper accumulated in juvenile trout exposed to copper for 28 d was found in tissues other than the gills (D. J. Laurén and D. G. McDonald, unpubl. data). Therefore, only a small portion of the copper accumulated during this slower phase of uptake actually stays in the gills.

We hypothesize that toxicity should be correlated with the actual amount of copper which enters the fish, rather than the apparent uptake which includes surface binding. If this is true, then the reduction of toxicity in high-alkalinity water must have been due to an increase in the portion of the whole body uptake attributable to surface binding (i.e. a reduction in uptake). Furthermore, the reduction in apparent uptake at pH 5.0, with no reduction in toxicity, must have been due to a decrease in the portion of the whole body uptake attributable to surface binding (i.e. an increase in actual uptake). In this respect, Miller and Mackay (1982) have shown that the copper binding capacity of mucus from rainbow trout is progressively reduced as pH is decreased, and it might be expected that mucus binding would be a major component of surface binding in fish. The other possible explanation for the apparent lack of correlation between uptake and toxicity is that the more toxic copper species, Cu^{2+}, was taken up at pH 5.0, while the nontoxic copper carbonates were taken up in HH. Longer-term experiments will be required to resolve these questions.

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Effects of pH on the Toxicities of Cadmium, Copper, and Zinc to Steelhead Trout (*Salmo gairdneri*)

Robert F. Cusimano and David F. Brakke

Institute for Watershed Studies, Western Washington University, Bellingham, WA 98225, USA

and Gary A. Chapman

Environmental Research Laboratory - Corvallis, U.S. Environmental Protection Agency, Corvallis, OR 97333, USA

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Increased metal concentrations have been associated with freshwater acidification. Continuous-flow acute toxicity tests were conducted in soft water to determine the effect of pH on the toxicity of cadmium, copper, and zinc to small (1–6 g) steelhead trout (*Salmo gairdneri*). LC50 values were calculated for 96- and 168-h exposure periods in waters of pH 4.7, 5.7, and 7.0. Test fish were significantly more tolerant of the metals at the lowest pH value than at higher pH's. The 96-h LC50 values at pH 4.7, 5.7, and 7.0 were 671, 97, and 66 $\mu g \cdot L^{-1}$ for zinc, 66.0, 4.2, and 2.8 $\mu g \cdot L^{-1}$ for copper, and 28.0, 0.7, and <0.5 $\mu g \cdot L^{-1}$ for cadmium, respectively. The 168-h results were similar to the 96-h values. These results indicate that for the metals tested, toxicity is ameliorated in depressed pH waters over short exposure periods, such as may occur during snowmelt runoff. The possibility of hydrogen ion interference with metal uptake is postulated.

L'augmentation de la concentration des métaux a été associé à l'acidification de l'eau douce. Des épreuves de toxicité aiguë, en flux continu, ont été exécutées en eau douce, afin de déterminer l'effet du pH sur la toxicité du cadmium, du cuivre et du zinc pour la petite truite (1 à 6 g) arc-en-ciel (*Salmo gairdneri*). Les valeurs de la CL50 ont été déterminées pour des périodes d'exposition de 96 et de 168 h dans des eaux de pH 4,7, 5,7 et 7,0. Les poissons étudiés ont montré une plus grande tolérance envers les métaux au pH le plus faible qu'au pH le plus élevé et cette différence a été d'importance significative. Après 96 h, les valeurs de la CL50 à pH 4,7, 5,7 et 7,0 étaient respectivement de 671, 97 et 66 $\mu g \cdot L^{-1}$ pour le zinc, 66,0, 4,2 et 2,8 $\mu g \cdot L^{-1}$ pour le cuivre et 28,0, 0,7 et $<0,5$ $\mu g \cdot L^{-1}$ pour le cadmium. Après 168 h, les valeurs ont été du même ordre que celles obtenues après 96 h. Ces résultats indiquent que la toxicité des métaux étudiés est moins grande dans les eaux de pH faible, pendant de courtes périodes d'exposition, comme c'est le cas au cours de l'écoulement des eaux de fonte. On peut supposer que l'ion d'hydrogène nuit à la captation des métaux.

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Increased availability of some metals in waters with low pH levels may result from (1) increased loading of metals from atmospheric deposition, (2) increased metal release rates from or to aquatic ecosystems due to deposition of acidifying components, and (3) changes in aqueous speciation of metals.

A number of factors are common to both metal and pH toxicities. In fish, gill tissue is considered the primary target organ for both acid stress (Beamish 1972; Daye and Garside 1976; Leivestad and Muniz 1976) and stress imposed by some metals (Phillips and Russo 1978; Alabaster and Lloyd 1980). Exposure to metals and increased H^+ concentrations are also associated with excess mucous secretion (Daye and Garside 1976; McDonald 1983; Alabaster and Lloyd 1980). Increased calcium levels or water hardness play an important role by reducing the impact of both these perturbants (Lloyd 1965; Wright and Snekvik 1978; Miller and Mackay 1980; Alabaster and Lloyd 1980; Brown 1981, 1982).

The toxic action of metals seems to be a combined effect of osmoregulatory stress and precipitation of mucus on the gills with death being attributed to suffocation. Zinc, copper, and cadmium cause necrotic damage in the gill epithelium (Alabaster and Lloyd 1980). Also, fish accumulate only small amounts of mucus on their gills when exposed to lethal levels of zinc (Lloyd 1960; Mount 1966). Therefore, the primary mode of action of toxic metals may be the disruption of gas exchange and salt/water balance caused by necrotic damage. Mucus accumulation on gill filaments would be of secondary importance.

Pagenkopf (1983) proposed a gill surface interaction model to explain the differences among experimental results in trace-metal toxicity to fish. He presents a model which explains metal toxicity with respect to metal complexation, pH, and water hardness. The purpose of this study was to examine the effect of acid pH on the toxicity of cadmium, copper, or zinc to steelhead trout (*Salmo gairdneri*). A softwater matrix was chosen to typify acid-sensitive aquatic systems.

Materials and Methods

Test Conditions

Each metal was tested simultaneously at three pH levels

TABLE 1. Mean water quality characteristics ($mg \cdot L^{-1}$) of well water, based on historical weekly sample data of the Environmental Research Laboratory, Corvallis, and analytical values for the blend water used in experiments. Blank values for the blend water are two to four times less than the well water.

Water quality character	Well water	Blend: well water + reverse osmosis water
Alkalinity	24	11.0[a]
Ammonia	0.04	
Cadmium	<0.001	<0.0005
Calcium	6.68	2.3
Chloride	6.0	2.1
Chromium	<0.002	
Cobalt	<0.002	
Copper	0.003	<0.0025
Dissolved solids	59	
Hardness	25	9.2[a]
Iron	0.098	0.04
Lead	0.004	
Magnesium	1.8	0.7
Manganese	0.004	0.002
Mercury	0.001	
Nickel	0.002	
Nitrate	0.19	0.10
Organic carbon	1.3	
Potassium	0.6	
Sodium	5.0	
Sulfate	4.2	4.6
Suspended solids	1.5	
Zinc	0.005	<0.005

[a] $mg \cdot L^{-1}$ as $CaCO_3$.

(4.7, 5.7, and 7.0), and each test was later repeated. Prior to each test, approximately 450 laboratory-reared steelhead fry, obtained from the same stock tank, were evenly divided among three outdoor holding tanks and held for 6 d at test pH levels of 4.7, 5.7, and 7.0. These tanks provided flowing, pH-adjusted water, a natural photoperiod, temperatures of 14.5–17.5°C, and dissolved oxygen greater than 90% saturation.

An acute exposure test required 120 fish from the appropriate pH tank, with 10 fish for each of six duplicated metal concentrations. The testing and holding period lasted 15 d: 6 d holding in the outdoor tanks, 2 d holding in the testing gear after transfer, and 7 d for each metal toxicity test. Fish were fed daily for the first 5 d of holding and not fed for the remaining 9 d.

Tests were conducted in 19-L glass aquaria, each containing a volume of approximately 14 L of water. The aquaria were dosed by proportional diluters (Mount and Brungs 1967) that delivered 2 L per cycle for each of the six metal concentrations. Three diluters were used, each with a water of predetermined pH (4.7, 5.7, and 7.0). More than 20 volumes of water were replaced in each aquaria during every 24-h period of each test. The replacement times were well within recommended limits (U.S. Environmental Protection Agency 1975).

Water Chemistry

It was necessary to simulate naturally occurring acid-sensitive waters that are very soft, have low dissolved solids, and interact minimally with metals. The water in which fish were held during toxicity tests and holding periods was a mixture of reverse osmosis (R.O.) treated well water and ambient well water that resulted in a mean EDTA hardness of 9.2 $mg \cdot L^{-1}$ as $CaCO_3$ (range = 8.0–11.0, $n = 42$) for all six toxicity series. Table 1 lists the mean water quality data for the well water supplied to the fish stock tank and for the test water.

During the test period, water hardness, alkalinity, and dissolved oxygen were monitored daily from samples taken from at least one aquarium containing the high metal concentration and one control from each diluter. Dissolved oxygen, hardness, and alkalinity measurements were made according to methods described in American Public Health Association (1980). In addition, alkalinity and pH were verified at least once during each test by the Gran (1952) plot titration method. No significant differences in hardness, alkalinity, or dissolved oxygen were seen between controls and metal-dosed aquaria at each pH. Dissolved oxygen was always greater than 90% saturated, and alkalinity varied from 11.0 ± 0.9 $mg \cdot L^{-1}$ as $CaCO_3$ at pH 7.0 to 1.7 ± 0.7 and −0.2 ± 0.6 $mg \cdot L^{-1}$ as $CaCO_3$ (±SD, $n = 42$) at pH values of 5.7 and 4.7, respectively. Water temperature was recorded continuously during all toxicity tests with test means of 15–16°C and a range of 14.5–17.0°C with most variation due to diel cycles.

Reagent grade H_2SO_4 was added to the R.O.-blend water to achieve pH 4.0, and then this solution was diluted with R.O.-blend water to reach target pH values. Actual pH values for the tests were 6.96 ± 0.09, 5.68 ± 0.09, and 4.67 ± 0.10 (±SD, $n = 42$). The pH was measured with an ORION 801/digital pH meter coupled with an ORION Ross-electrode 81-02.[1] An automatic titrator was used to acidify the pH 4 tank, and a Beckman model 942 pH monitor and combination electrode provided continuous monitoring/control.

Sulfate levels increased from 4.6 $mg \cdot L^{-1}$ at pH 7.0 to 8.6 and 11.3 $mg \cdot L^{-1}$ at pH 5.7 and 4.7, respectively. Stock metal solutions of reagent grade $CuCl_2 \cdot 2H_2O$, $CdCl_2 \cdot 2\frac{1}{2}H_2O$, and $ZnCl_2$ were prepared with ambient well water and were acidified by the addition of 0.1 mL $HNO_3 \cdot L$ stock solution^{-1} to assure total dissolution.

Metal Analysis

Metal samples were collected daily during each test from one of each of the duplicate metal containing aquaria and one of the controls. Metal concentrations were determined using a Perkin-Elmer 305B atomic absorption spectrophotometer. The flame method was used for zinc and the flameless method for cadmium and copper. Calcium and magnesium were measured using a flame atomic absorption spectrophotometer (Perkin-Elmer 560) and nitrate and sulfate using a Scientific CFA200 autoanalyzer.

Statistical Analysis

Following the initial toxicity test with cadmium, a second cadmium test was conducted, followed by two successive zinc toxicity tests, and concluding with two copper toxicity tests. Dead fish were removed and recorded every 12 h during all tests. For each metal and pH, pooled data from the two consecutive acute tests were used to calculate the LC50 values. Calculations of 96- and 168-h LC50 values and corresponding confidence limits were made using the 10% trimmed Spearman–Karber method (Hamilton et al. 1977). This method is model free, requiring only the symmetry of the cumulative relative

[1] Mention of trade names does not constitute endorsement by the U.S. Environmental Protection Agency.

TABLE 2. Fish number, weight, length, and condition factor (=wt (g) × 100/L^3 (cm^3)) for fish stock and control fish from all tests after the 168-h testing period

Source	n	Wet weight (g) Mean	Wet weight (g) SD	Fork length (cm) Mean	Fork length (cm) SD	Condition factor[a] Mean	Condition factor[a] SD
Stock	30	3.36	1.06	7.0	0.8	0.91	0.06
Controls							
pH 4.7	116	2.88	1.08	6.9	0.8	0.85	0.06
pH 5.7	119	2.70	1.10	6.8	0.9	0.83	0.06
pH 7.0	118	2.65	0.93	6.7	0.7	0.84	0.06

[a]Stock fish condition factor is significantly different from condition factors of control fish (results of Student–Newman–Keuls multiple range test on condition factors).

frequency curve for the tolerance distribution. Abbott's formula (Finney 1971) was used for correcting mortality data for control mortality.

Wet weight and fork length of control fishes were measured at the end of each testing period. These data were used to calculate condition factors for fish exposed to the different test pH values. Condition factors for control fish from each pH and the stock tank fish were compared using the Student–Newman–Keuls test.

Results

Mean wet weight of fish over the duration of the study ranged from 2.36 to 3.01 g. Growth of stock fish during the 3-mo testing period was minimal and probably did not influence test results. Test fish were less fit, judged by the condition factors (Table 2), after the holding and test periods than the stock fish. Factors that possibly contribute to the difference are (1) test fish were not fed for 9 d and (2) the low ionic strength of the test waters may have introduced extra osmoregulatory stress. However, there was no difference in condition factor among pHs.

The 96- and 168-h LC50 values and their 95% confidence intervals from the toxicity tests are presented in Table 3. These values were calculated from 5–10 different exposure concentrations from the pooled replicate toxicity tests. Mortality in the control tanks averaged <2% and never exceeded 10% for all tests. The LC50 value for cadmium at pH 7.0 could not be differentiated from the background cadmium levels because all but the lowest exposure concentration of 0.4 $\mu g \cdot L^{-1}$ (detection limit 0.5 $\mu g \cdot L^{-1}$) produced total mortality.

The 168-h test period was sufficient to attain an asymptotic LC50 value for all metals at pH levels of 5.7 and 7.0 (Fig. 1a–1c). The curves demonstrate that a threshold of acute lethality was reached at these pH values. However, this period was not sufficient to reach a threshold at pH 4.7. Although a steady state is not achieved in the pH 4.7 water, the LC50 data and corresponding curves reflect the existence of a pronounced antagonist effect of low pH on the acute toxicity of the metals tested.

Discussion

The unusually low LC50 concentrations at pH 7.0 and 5.7 may serve to emphasize the importance of calcium when determining water quality criteria. Chapman (1978), working with swim-up steelhead in the same testing system, established 96-h

TABLE 3. Calculated 96- and 168-h LC50 values ($\mu g \cdot L^{-1}$, mean and 95% confidence limits) for steelhead exposed to zinc, cadmium, or copper in waters with pH values of 4.7, 5.7, and 7.0.

Metal and pH	96-h LC50	168-h LC50
Zinc		
4.7	671(908–497)	501(652–385)
5.7	97(112–84)	97(112–84)
7.0	66(79–56)	66(79–56)
Cadmium		
4.7	28(37–22)	6.3(8.9–4.6)
5.7	0.7(0.8–0.6)	0.7(0.8–0.6)
7.0	<0.5	<0.5
Copper		
4.7	66.0(74.7–58.3)	36.7(41.7–32.5)
5.7	4.2(5.2–3.4)	3.1(3.7–2.5)
7.0	2.8(3.8–2.0)	2.3(3.0–1.7)

LC50 values for cadmium, copper, and zinc of 1.3, 17, and 93 $\mu g \cdot L^{-1}$, respectively, at a median pH of 7.1. These results were obtained in test waters with a calcium concentration of 6.1 $mg \cdot L^{-1}$. The much lower LC50 values reported in this paper for pH 7.0 reflect responses to conditions of significantly lower calcium levels of 2.3 $mg \cdot L^{-1}$.

Other researchers have noted changes in metal toxicity with pH. Decreased toxicity of copper with increasing H^+ concentrations was reported by Miller and Mackay (1980). They found copper to be more toxic to rainbow trout at a pH of 5.4 than at 4.7. Howarth and Sprague (1978) noted that thresholds of acute lethality were reached within 72 h for most pH values and hardness levels tested; however, lower pH values seldom reached thresholds by 5 d of exposure. They reported copper to be more toxic at pH 6.0 than at pH 5.0 for most hardness levels, with the greatest toxicity occurring at a neutral pH. It has also been suggested that zinc follows this same pattern (Alabaster and Lloyd 1980).

The ameliorating effect of low pH on metal toxicity may be due to (1) stimulated mucous secretions due to H^+, with the resulting free mucus able to chelate the metals, (2) changes in toxic species of metals with the test pH, and/or (3) competitive inhibition of Me^{2+} by H^+ at gill binding sites. These hypothesis are treated below. A fourth possibility not subject to treatment with our data is proton-induced steric modification of binding sites.

McDonald (1983) pointed out that under normal circumstances the gills of freshwater fish are covered with a thin layer of mucus. This layer consists of a water-soluble complex of glycoproteins which has a net negative charge at neutral pH. He further stated that acidification of the medium will titrate the charged residues and could interfere with their ion binding properties. Chelation of metals by proteinaceous mucus probably occurs at all pH levels given that stimulation of mucus secretion is a response to both metal and acid exposure. One of the functions of the flow-through testing apparatus was to minimize collection of organic material in the testing chambers. Each test aquarium passed more than 280 L water $\cdot d^{-1}$ while containing approximately 30 g of fish. This excludes the possibility of significant accumulation of mucus in the water matrix. Miller and McKay (1982) showed that a fish mucous solution, which binds copper at neutral pH, loses a significant proportion of that binding capacity at pH 4.5, and at pH 3.5 loses binding

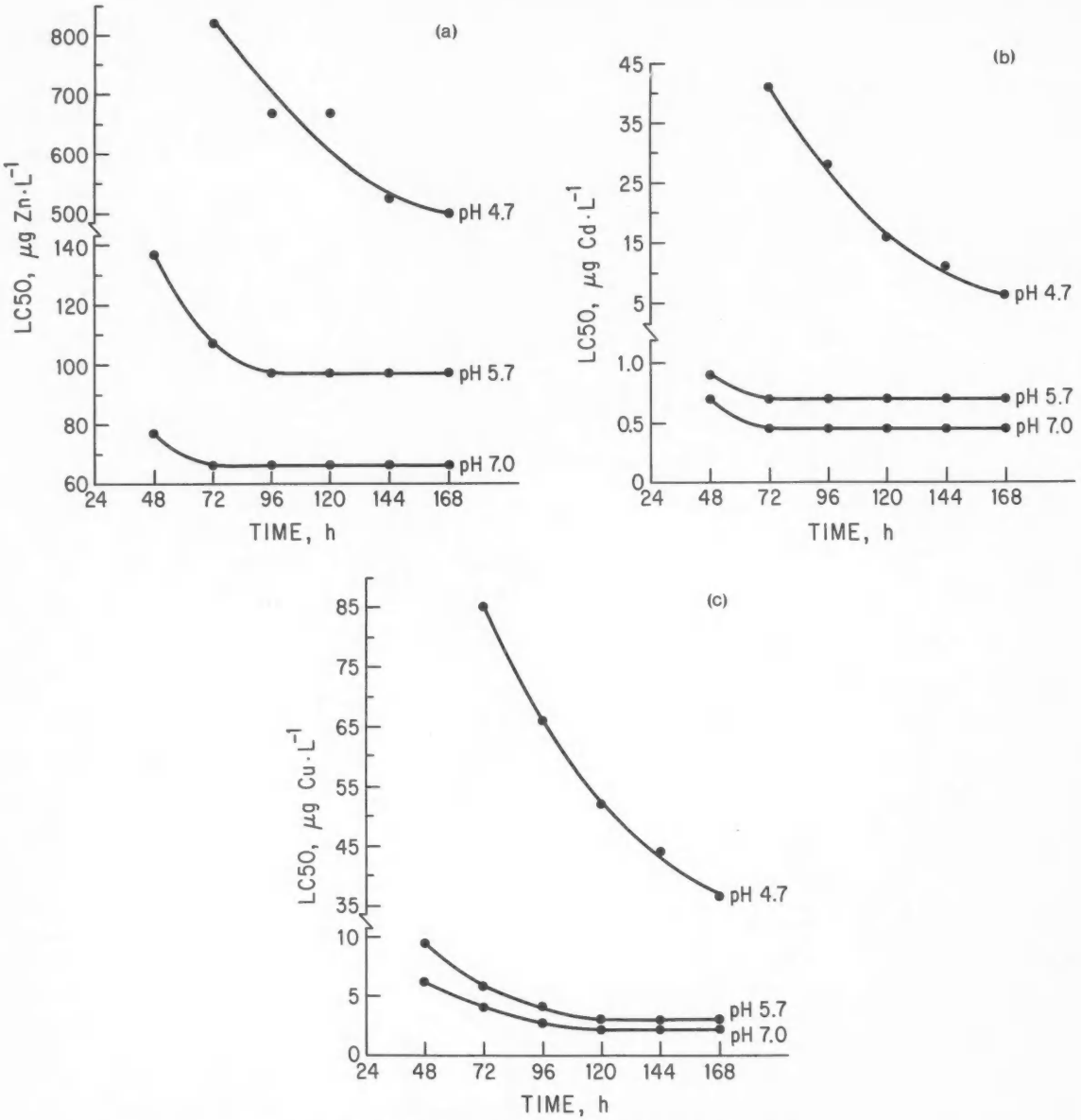

FIG. 1. Toxicity curves for (a) zinc, (b) cadmium, and (c) copper.

ability entirely. Loss of metal binding capacity of mucus at acid pH actually is more significant than the increase in mucous production, so that the net effect is to reduce metal binding to mucus as compared with neutral pH values.

Changes in metal speciation might have accounted for the observed difference in toxicity. Speciation of the metals is dependent upon pH and the presence of complexing ligands. Sulfate and alkalinity varied with pH, and other known ligands (Cl^-, NO_3^-) remained constant. The ionic strength calculated for the most concentrated test water was 9.6×10^{-4} *M*. Ions were therefore assumed to have activity coefficients of unity and equilibria constants were not corrected for ionic strength (Stumm and Morgan 1981). Equilibrium reactions used are listed in Table 4. The equilibrium relationships of these reactions were evaluated by a computer model developed by Westall (1979). Table 5 presents the proportions of the four most abundant metal species at each of the test pH values for a total metal concentration equal to the calculated 96-h LC50. According to model predictions, major metal species changes did not occur. Consequently, changes in metal speciation did not have a significant role in the toxic effect of the metals tested. The test waters were dominated by the free ion, with

TABLE 4. Equilibrium reactions and stability constants of the various metal species considered in the Westall (1979) model at infinite dilution (data from Smith and Martell (1976) except as indicated).

Reaction	log K_{eq}
$Cu^{2+} + HCO_3^- = CuHCO_3^+$	2.7[a]
$Cu^{2+} + H_2O = Cu(OH)^+ + H^+$	−7.7
$Cu^{2+} + 2H_2O = Cu(OH)_2 + 2H^+$	−14.32
$2Cu^{2+} + 2H_2O = Cu_2(OH)_2^{2+} + 2H^+$	−10.3
$Cu^{2+} + Cl^- = CuCl^+$	0.4
$Cu^{2+} + SO_4^{2-} = CuSO_4$	2.36
$Cu^{2+} + NO_3^- = CuNO_3^+$	0.5
$Cu^{2+} + 2NO_3^- = Cu(NO_3)_2$	−0.4
$Cd^{2+} + HCO_3^- = CdHCO_3^+$	1.8[a]
$Cd^{2+} + H_2O = Cd(OH)^+ + H^+$	−10.1
$Cd^{2+} + 2H_2O = Cd(OH)_2 + 2H^+$	−20.4
$2Cd^{2+} + H_2O = Cd_2(OH)^{3+} + H^+$	−9.3
$Cd^{2+} + Cl^- = CdCl^-$	1.98
$Cd^{2+} + SO_4^{2-} = CdSO_4$	2.46
$Cd^{2+} + NO_3^- = CdNO_3^+$	0.5
$Cd^{2+} + 2NO_3^- = Cd(NO_3)_2$	0.2
$Zn^{2+} + HCO_3^- = ZnHCO_3^+$	1.9[a]
$Zn^{2+} + H_2O = Zn(OH)^+ + H^+$	−8.96
$Zn^{2+} + 2H_2O = Zn(OH)_2 + 2H$	−16.9
$Zn^{2+} + 3H_2O = Zn(OH)_3^- + 3H^+$	−28.4
$Zn^{2+} + Cl^- = ZnCl^+$	0.43
$Zn^{2+} + SO_4^{2-} = ZnSO_4$	2.38
$Zn^{2+} + NO_3^- = ZnNO_3^+$	0.4
$Zn^{2+} + 2NO_3^- = Zn(NO_3)_2$	−0.3

[a]From Mantoura et al. (1978).

only copper at pH 7.0 having substantial percentages of other species. Calcium speciation was also considered in this model, and the Ca^{2+} form was calculated to be >97% of the total calcium for all pH levels. Thus the delayed toxicity of the Me^{2+} at pH 4.7 apparently did not stem from speciation changes of metals or changes in available calcium.

Hydrogen ion interference with metal uptake at the gills is proposed as the major mechanism that decreased the apparent toxicity of metals at acid pH. Fish gills are known to have a net negative charge in positive ion transport channels that is decreased following exposure to low pH (McDonald 1983). Uptake of cations such as calcium and the toxic metals might involve loose binding to negatively charged gill uptake sites and transport through channels lined with similar anionic sites. High concentrations of H^+ could neutralize the anionic gill sites, sites behaving as weak acids, the dissociation of which is governed by H^+ concentration and the pK of the weakly acidic sites:

$$\% \text{ ionized} = \frac{1}{1 + 10^{(pK - pH)}}.$$

A theoretical mechanism that appears to fit the data assumes essentially zero-order uptake of the metal ions. According to this model, isotoxic concentrations of the metals are inversely proportional to the percentage of anionic (nonprotonated) gill sites:

TABLE 5. Metal speciation for 96-h LC50 values and corresponding percentage of total metal concentration (Me_T).

pH 7.0	% of Me_T	pH 5.7	% of Me_T	pH 4.7	% of Me_T
Cu^{2+}	71	Cu^{2+}	95	Cu^{2+}	97
$Cu(OH)_2$	11	$CuSO_4$	2	$CuSO_4$	3
$Cu(OH)^+$	8	$CuHCO_3^+$	2	$Cu(OH)^+$	<1
$CuHCO_3^+$	8	$Cu(OH)^+$	<1	$CuCl^-$	<1
Cd^{2+}	97	Cd^{2+}	97	Cd^{2+}	96
$CdSO_4$	1	$CdSO_4$	2	$CdSO_4$	3
$CdHCO_3^+$	1	$CdCl^+$	<1	$CdCl^+$	<1
$CdCl^+$	<1	$CdHCO_3^+$	<1	$Cd(OH)^+$	<1
Zn^{2+}	96	Zn^{2+}	97	Zn^{2+}	97
$ZnHCO_3^+$	2	$ZnSO_4$	2	$ZnSO_4$	3
$ZnSO_4$	1	$ZnHCO_3^+$	<1	$ZnCl^+$	<1
$Zn(OH)^+$	<1	$Zn(OH)^+$	<1	$Zn(OH)^+$	<1

$$\frac{LC50_1}{LC50_2} = \frac{\% \text{ ionized}_2}{\% \text{ ionized}_1} = \frac{1 + 10^{(pK - pH_1)}}{1 + 10^{(pK - pH_2)}}.$$

A theoretical pK value (5.4) for gill binding sites was calculated from the zinc toxicity data at pH 7.0 and 5.7 (where mortality had stabilized as indicated by the identical 96- and 168-h values). Using this pK value the incipient zinc LC50 value was calculated for pH 4.7. Similar calculations were performed with this pK value for the cadmium and copper data, basing each calculation on the most temporally stable LC50 estimates (for cadmium, pH 5.7; for copper, pH 7.0).

The pattern of LC50 values observed with cadmium, copper, and zinc is approximated by the model predictions (Fig. 2) although the estimates for cadmium and copper at pH 4.7 are significantly below observed values. The LC50 values for cadmium and copper at pH 4.7 dropped considerably between 96 and 168 h, and incipient LC50 values might have approached predicted levels had the tests lasted longer than 168 h.

Response of metal toxicity to pH over the range seen in freshwater (4−9) is influenced by aqueous metal speciation and competition for cation binding/uptake sites on the gill surface. In low ionic strength, acid waters, competition is the major feature, with hydrogen playing the overwhelmingly predominant role in extremely soft, acid waters. At pH values where the weakly acid binding/uptake sites are not significantly influenced by pH (i.e. pH > pK + 1), two other factors become dominant. First, pH values on the basic side of neutrality usually characterize water with higher alkalinity, with both hydroxides and carbonates capable of complexing the metal ions. Second, increased pH and alkalinity are usually indicative of increased hardness, with either calcium or magnesium or both competing with toxic metals for binding/uptake sites. As a result, maximum acute toxicity of many metals will occur at slightly acid to neutral pH.

Our results and theory suggest several areas of further investigation: (1) longer acute response studies at the more acid pH values to elucidate the asymptotic LC50, (2) chronic metal toxicity studies in acid waters to see if the ameliorating effect of acid pH extends to long-term metal exposure, and (3) exposure of fish to metals in low ionic strength, alkaline waters, an atypical matrix that would allow estimation of the toxicity of metal hydroxides in isolation from significant carbonate speciation or hardness ion competition.

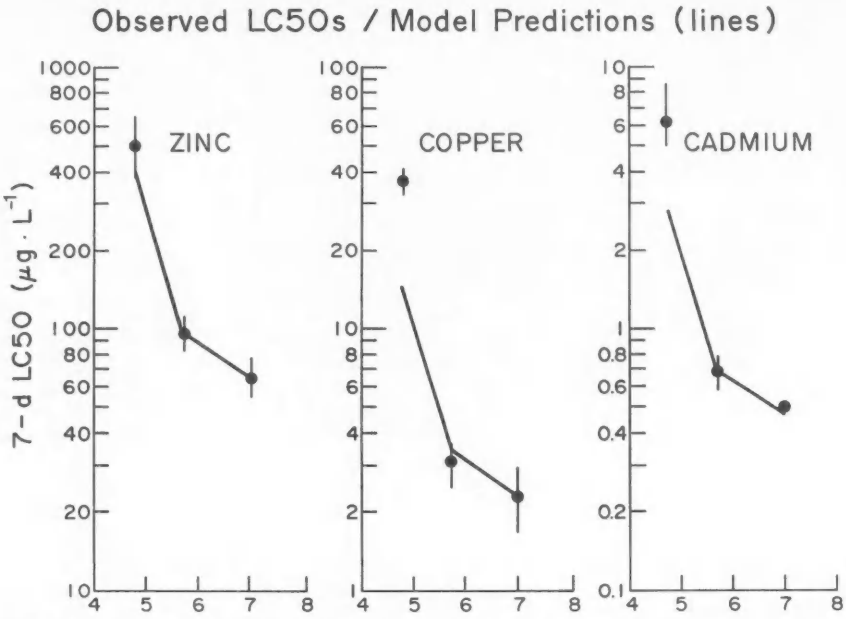

FIG. 2. Comparison of effects of pH on observed 168-h LC50 concentrations compared with the model-predicted pH effect.

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Bacterioplankton, Phytoplankton, and Zooplankton Communities in a British Columbia Coastal Lake Before and After Nutrient Reduction

F. Joan Hardy, Ken S. Shortreed, and John G. Stockner

Department of Fisheries and Oceans, Fisheries Research Branch, West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, B.C., V7V 1N6

Hardy, F. J., K. S. Shortreed, and J. G. Stockner. 1986. Bacterioplankton, phytoplankton, and zooplankton communities in a British Columbia coastal lake before and after nutrient reduction. Can. J. Fish. Aquat. Sci. 43: 1504–1514.

Inorganic nitrogen and phosphorus were applied weekly during the growing season from 1980 to 1982 and twice weekly in 1983 to Hobiton Lake, a warm monomictic coastal lake in British Columbia. The lake was not fertilized in 1984. Average numbers of bacteria during the growing season decreased from a high of $1.53 \times 10^6 \cdot mL^{-1}$ in the fertilized condition to $0.84 \times 10^6 \cdot mL^{-1}$ in the unfertilized condition. Chlorophyll *a* concentrations decreased from a maximum seasonal average of $2.69\ \mu g \cdot L^{-1}$ (1981) to $1.30\ \mu g \cdot L^{-1}$ (1984), and algal numbers decreased from $5.83 \times 10^4 \cdot mL^{-1}$ (1983) to $2.29 \times 10^4 \cdot mL^{-1}$ (1984). Although the numbers of phytoplankton in each size fraction (picoplankton, nanoplankton, or microplankton) decreased in the unfertilized condition, the greatest change was an almost fourfold decrease in picoplankton, which consisted of 90% cyanobacteria (primarily *Synechococcus* spp.). Abundance of the large diatoms *Rhizosolenia* spp. and *Melosira* spp. increased in 1984, resulting in an increase in average seasonal algal volume. Average densities of medium (0.15–0.84 mm) and large (0.85–1.5 mm) zooplankton were greatest in 1982, while rotifers and small zooplankton (0.10–0.14 mm) were most dense in 1984 following nutrient reduction. The lake had relatively high concentrations of planktivorous juvenile sockeye salmon (*Oncorhynchus nerka*) that appeared to minimize any direct effect of nutrient additions on zooplankton densities.

De l'azote et du phosphore inorganiques ont été ajoutés, pendant la période de croissance, une fois par semaine, de 1980 à 1982 et deux fois par semaine en 1983 à l'eau du lac Hobiton, lac côtier monomictique de Colombie-Britannique. Le lac ne fut pas fertilisé en 1984. La population bactérienne moyenne au cours de la saison de croissance a diminué de $1,53 \times 10^6\ mL^{-1}$, en présence de fertilisation, à $0,84 \times 10^6\ mL^{-1}$, en l'absence de fertilisation. Les concentrations de chlorophylle *a* sont passées de la moyenne maximale saisonnière de 2,69 $\mu g \cdot L^{-1}$ (1981) à 1,30 $\mu g \cdot L^{-1}$ (1984), et le nombre des algues de $5,83 \times 10^4\ mL^{-1}$ (1983) à $2,29 \times 10^4\ mL^{-1}$ (1984). Bien que le nombre des phytoplanctons de chaque taille (picoplancton, nanoplancton ou microplancton) ait diminué en l'absence de fertilisation, le plus important changement a été une diminution au quart des picoplanctons comprenant 90 % de cyanobactéries (principalement des *Synechococcus*) en comparaison avec la situation de l'année précédente. Une abondance des diatomées géantes des espèces *Rhizosolenia* et *Melosira*, dont le nombre a augmenté en 1984, a amené un accroissement du volume moyen saisonnier des algues. Les densités moyennes du zooplancton de tailles moyenne (0,15–0,84 mm) et grande (0,85–1,5 mm) ont été les plus importantes en 1982 alors que les rotifères et le zooplancton de petite taille (0,10–0,14 mm) ont connu une plus forte densité en 1984 suite à l'appauvrissement de nutriments. Le lac contenait des concentrations relativement élevées de saumons sockeye juvéniles (*Oncorhynchus nerka*) planctonophages qui ont paru minimiser les effets directs des additions de nutriments sur la densité de la population de zooplankton.

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Hobiton Lake is one of numerous coastal oligotrophic lakes in British Columbia in which low phosphorus and nitrogen concentrations limit primary production and phytoplankton biomass (Stockner et al. 1980; Stockner 1981; Stockner and Shortreed 1985). Addition of inorganic phosphorus and nitrogen fertilizers to these lakes to increase primary and secondary production is now an established sockeye salmon (*Oncorhynchus nerka*) enhancement technique in British Columbia (Stephens and Stockner 1983; Stockner and Hyatt 1984; Stockner and Shortreed 1985) that has resulted in increased growth of underyearling sockeye salmon (Hyatt and Stockner 1985).

The importance of picoplankton and nanoplankton in microbial food webs has recently been observed in oceanic and freshwater phytoplankton communities (e.g. Paerl 1977; Booth et al. 1982; Li et al. 1983; Platt et al. 1983; Davis et al. 1985). The size–frequency of the phytoplankton community is important to the herbivorous zooplankton community (Burns 1968; Gliwicz 1975; Porter 1977; Vanderploeg 1981) and thus may affect the food resources available to planktivorous juvenile sockeye salmon. If nutrient uptake rates are size dependent (e.g. Eppley et al. 1969; Laws 1975; Smith and Kalff 1982;

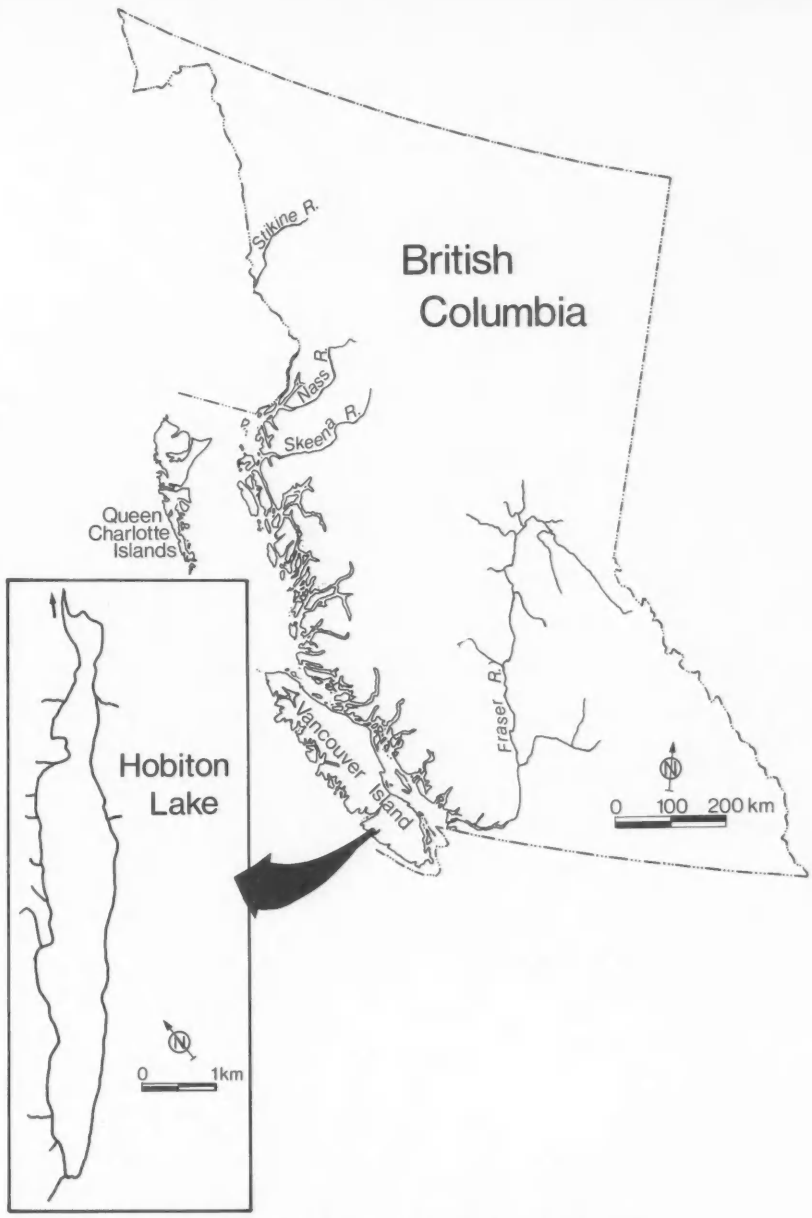

FIG. 1. Hobiton Lake showing its location in British Columbia.

Lehman and Sandgren 1982), nitrogen and phosphorus levels as well as alterations in the ambient N:P ratio may also influence the size spectrum of the phytoplankton. Although detailed effects of lake fertilization on periphyton communities (Shortreed et al. 1984) and on zooplankton (LeBrasseur and Kennedy 1972; Hyatt and Stockner 1985) in selected British Columbia coastal lakes have been reported, effects on the pelagic bacteria and phytoplankton communities of these lakes have not been discussed in detail. Objectives of this study were twofold: (1) to describe the size–frequency, abundance, and distribution of the pelagic phytoplankton community in a fertilized coastal lake and (2) to determine changes in the bacterioplankton, phytoplankton, and zooplankton communities the year immediately following termination of fertilization.

Description of the Study Lake

Hobiton Lake is located near the British Columbia coast on Vancouver Island (48°45′N, 124°49′W) (Fig. 1). It is a clear and warm monomictic lake with a surface area of 3.6 km^2, a mean depth of 36.0 m, and water residence time of 1.0 yr. The drainage basin features rugged terrain from sea level to 700 m

TABLE 1. Seasonal averages of salient physical and chemical variables for Hobiton Lake, 1980–84.

Year	Surface temperature (°C)	Compensation depth (m)	Epilimnion depth (m)	Epilimnetic total nitrogen ($\mu g \cdot L^{-1}$)	Epilimnetic nitrate ($\mu g \cdot L^{-1}$)	Epilimnetic total phosphorus ($\mu g \cdot L^{-1}$)	Epilimnetic particulate phosphorus ($\mu g \cdot L^{-1}$)	TN:TP (weight)	Fertilizer phosphorus load ($mg \cdot m^{-2} \cdot wk^{-1}$)	Annual phosphorus load (fertilizer) ($mg \cdot m^{-2} \cdot yr^{-1}$)	Soluble reactive silicon ($mg \cdot L^{-1}$)
1980	15.5	7.7	9.0	—	3.0	2.2	—	—	3.86	88.78	0.96
1981	15.7	7.5	8.4	154	4.6	3.6	2.6	52:1	3.95	102.70	1.01
1982	17.8	9.0	7.7	159	11.7	3.9	2.9	38:1	3.97	83.37	0.91
1983	16.4	7.2	7.8	276	2.0	3.2	2.4	59:1	3.93	70.74	0.89
1984	15.5	12.7	8.0	229	6.0	2.1	1.8	129:1	0	0	1.01

elevation with steep slopes, and the forest consists of old-growth western hemlock (*Tsuga heterophylla*), amabilis fir (*Abies amabilis*), and western red cedar (*Thuja plicata*) with some Douglas-fir (*Pseudotsuga menziesii*), Sitka spruce (*Picea sitchensis*), and red alder (*Alnus rubra*) (Hetherington 1982). The climate of the watershed is influenced by the adjacent Pacific Ocean and features mild wet winters with high annual rainfall (>200 cm) (Stockner et al. 1980). Hobiton Lake has low inorganic nutrient levels (Table 1), low bacteria and phytoplankton biomass (MacIsaac et al. 1981; Shortreed and Stockner 1981), and low zooplankton biomass (Rankin et al. 1979; Rankin and Ashton 1980). Hobiton Lake is a sockeye nursery lake and has an adult sockeye salmon escapement ranging from <2000 to 50 000 sockeye $\cdot yr^{-1}$.

Methods

Hobiton Lake was fertilized once (1980–82) or twice (1983) weekly with an aqueous solution of ammonium nitrate and ammonium phosphate in an N:P atomic ratio of 15:1 (Table 1). Fertilizer was applied using a DC-6B water bomber for a period of 19–26 wk commencing in April–May (Stephens and Stockner 1983). Hobiton Lake was not fertilized in 1984.

A float-equipped DeHavilland Beaver aircraft was used to sample the lake once monthly from March–April to October–November (7–9 times $\cdot yr^{-1}$). Sampling took place 2–4 d after a fertilizer application. Results are reported as growing season averages, i.e. the period when surface temperatures reach 8°C in spring and decrease to 12°C in fall.

Temperature profiles to a depth of 50 m were obtained using a Montedoro–Whitney temperature probe (model TC-5C). Buoyancy frequencies (Turner 1973) were calculated from temperature data and used to determine epilimnion depth. A Li-Cor light meter (model 185A) equipped with an underwater quantum sensor (model 192S) was used to measure photosynthetically active radiation (400–700 nm) from the water surface to depths where light intensity was 1% of surface values. Vertical extinction coefficients and compensation depths were calculated using light data.

A 6-L, opaque Van Dorn bottle was used to obtain water samples from three epilimnetic and one hypolimnetic depths. Two 2-L polyethylene bottles and one glass culture tube were filled with water from each depth. Samples were preserved within 4 h of collection, and all subsequent chemical analyses were carried out at the Pacific Biological Station, Nanaimo, B.C., using the methods of Stephens and Brandstaetter (1983).

From each sampling depth, 2 L of water was filtered through a cleaned Whatman GF/F glass fiber filter which was then placed into a clean glass vial. Particulate phosphorus was determined from these samples after ignition, conversion to orthophosphorus by acid digestion, and detection by a manual molybdenum blue method. Total phosphorus was determined from water collected in the glass culture tubes by a molybdenum blue method after persulphate digestion (Traversy 1971).

Water from each depth was filtered through an ashed and rinsed (500 mL of distilled water and 200 mL of sample) Whatman GF/F filter and placed in both a glass and plastic bottle. Total dissolved solids were determined from water in the plastic bottles by evaporating the sample and weighing the residue (American Public Health Association 1980). Water from glass bottles was analyzed for nitrate and total dissolved

nitrogen. Ammonia was determined using the method described by Strickland and Parsons (1972) and modified for freshwater by Stephens and Brandstaetter (1983). Nitrate was converted to nitrite by cadmium reduction and concentration determined after the reaction which formed a colored azodye. Total dissolved nitrogen was oxidized by the ultraviolet (UV) oxidation method, then reduced to ammonia by passage through a zinc reduction column, and ammonia determined as indophenol blue (Stainton et al. 1977). Particulate nitrogen and carbon were determined by filtering 1 L of water from each sampling depth through an ashed and washed Whatman GF/F filter. Filters were dried in a desiccator, placed in foil dishes, frozen, and later analyzed using a Perkin Elmer model 240 elemental analyzer. Samples for chlorophyll *a* determinations were collected by filtering 0.5 L of water from each depth through 47-mm-diameter, 0.8-μm Millipore AA filter. Filters were placed in foil dishes, dried in a desiccator, and frozen. Samples were macerated in 90% acetone and analyzed using a Turner model III fluorometer.

Test tubes rinsed with 95% ethanol were rinsed and filled with water from 1, 3, 5, and 30 m for bacteria enumeration. In the field laboratory, 5 mL from each depth was filtered onto a 25-mm-diameter, 0.2-μm Nuclepore membrane filter counterstained with Irgalan black. Filters were removed when just dry, placed into a 9-cm divided petri dish lined with Whatman filter paper, air-dried at room temperature, and stored. Bacteria were later counted using the acridine orange direct count method as described by MacIsaac et al. (1981).Generally, 300 bacteria or 10 fields were enumerated and counts converted to numbers per millilitre.

Samples for phytoplankton enumeration and identification were collected from 1, 3, 5, and 30 m in opaque, 125-mL polyethylene bottles and transported to the field laboratory. In this study, picoplankton, nanoplankton, and microplankton are defined as having maximum dimensions of <3, $3-20$, and >20 μm, respectively. Beginning in 1981, picoplankton were counted according to methods described by MacIsaac and Stockner (1985). Samples were preserved by filtering 15 mL of each sample onto a counterstained (Irgalan black), 25-mm-diameter, 0.2-μm Nuclepore membrane filter in the same manner as for bacteria biomass. Filters were air-dried and stored in opaque, 9-cm petri dishes at room temperature until counted. Just prior to picoplankton identification and enumeration, each filter was placed on top of a piece of Nitex mesh in a Millipore filtering unit and rehydrated with $1-2$ mL of cold, filtered, distilled water for approximately 3 min. The water was then drawn through and the moist filter placed on a microscope slide, followed by a drop of Cargille type A immersion oil, a coverslip, and another drop of oil. Counts were done at 1250× magnification using a Zeiss compound microscope (model KLSM) equipped with a IV/FL epifluorescent condenser, HBO 50-W mercury lamp, LP 397 and KP 560 exciter filters, FT 580 beam splitter, and LP 590 barrier filter. Random fields were counted until a minimum of 200 cells or 30 fields were counted per sample. The remainder of each sample was fixed with 1 mL of Lugol's acid solution and used for enumeration of nano- and microplankton (>3 μm equivalent spherical diameter). Samples were shaken and settled overnight in 7-, 12- or 27-mL settling chambers. One transect at 187.5× and one at 750× magnification were counted using a Wild M40 inverted microscope equipped with phase contrast optics. Carbon content of the phytoplankton was calculated (Strathmann 1967) and expresses as milligrams carbon per cubic metre.

Zooplankton were collected at every station using a 100-μm mesh size SCOR-UNESCO net (mouth area = 0.25 m^2) which was hauled vertically at approximately 0.5 $m \cdot s^{-1}$ from 50 m to the surface. Zooplankton were preserved in a borax-buffered, 4% formalin−sucrose solution (Haney and Hall 1973). In the laboratory, each sample was halved using a Folsom plankton splitter. One portion was filtered onto an ashed and weighed Whatman GFC filter, dried to a constant weight at 90°C for 24 h, and weighed. These samples were then ashed (460°C for 4 h) and weighed again. Zooplankton biomass was expressed as mg dry weight per cubic metre. The other portion of the sample was used for zooplankton identification and enumeration using a microcomputer-based caliper system (Sprules et al. 1981).

Results

Physics and Chemistry of the Study Lake

Mean daily air temperature for the years 1980−84 exhibited little annual variation and was highest in August and lowest in December or January. Total monthly hours of sunshine were lowest from November through February and highest usually in July and August. Hours of sunshine during the spring displayed considerable annual variability; during 1982 and 1983, total hours of sunshine in spring were 32% greater than in other study years. Total monthly precipitation was variable between years but was always highest from November through February and lowest from May through September. Thus, greatest hydrologic flux and peak nutrient loading occurred in winter when low temperatures and low light intensities were observed and when considerable vertical mixing was taking place.

Seasonal (April−October) average epilimnion depths ranged from 7.7 to 9.0 m, and average growing season surface temperatures ranged from 15.5 to 17.8°C (Table 1). Stratification usually began in April and overturn usually occurred by mid-November. Results are reported as annual growing season averages.

Generally, nitrate levels were highest ($27-33$ μg $N \cdot L^{-1}$) in early spring, dropped below the analytical detection limit of 1 μg $N \cdot L^{-1}$ by mid-May, and increased by November (Fig. 2). Average concentrations of total nitrogen (TN) ranged from a low of 154 μg $\cdot L^{-1}$ in 1981 to 276 μg $\cdot L^{-1}$ in 1983 (Table 1). Average total phosphorus (TP) ranged from 2.2 (1980) to 3.9 μg $\cdot L^{-1}$ (1982) (Fig. 2), while particulate phosphorus ranged from 1.8 (1984) to 2.9 μg $\cdot L^{-1}$ (1982). The ratio of TN:TP was lower for most of the season in fertilized years than in the nonfertilized year (1984). Average annual silicon concentrations ranged from 0.89 to 1.01 mg $\cdot L^{-1}$ and concentrations throughout the season did not exhibit a period of depletion.

Bacteria

In fertilized years, average bacteria numbers ranged from 1.25×10^6 to $1.53 \times 10^6 \cdot mL^{-1}$ (Fig. 3). When nutrients were not added to Hobiton Lake (1984), the average number of bacteria dropped to $0.84 \times 10^6 \cdot mL^{-1}$. Numbers of bacteria in the spring during years of fertilization were usually low but increased by July, August, or September and then decreased in late fall. In the year without fertilization, numbers of bacteria did not increase above spring values until the fall overturn in October.

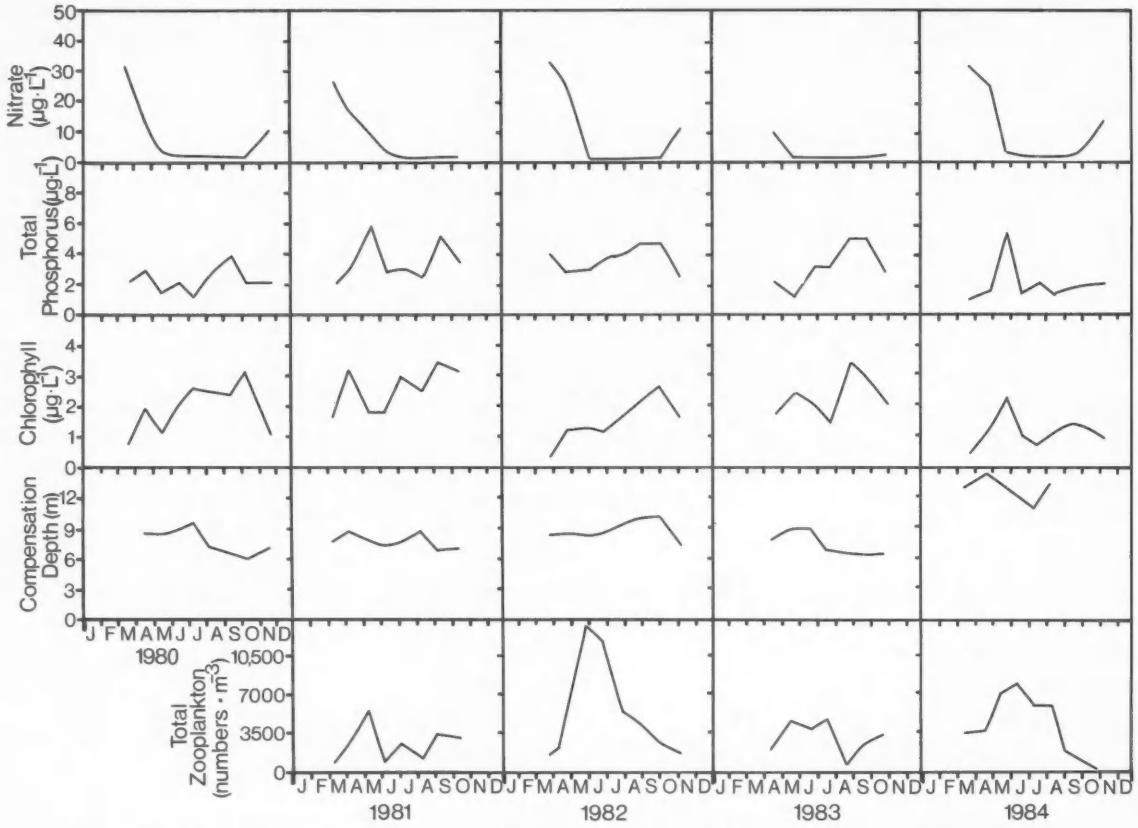

FIG. 2. Seasonal variation in nitrate-nitrogen, total phosphorus, chlorophyll, compensation depth, and zooplankton density.

Phytoplankton

Average concentrations of chlorophyll *a* and algal numbers were lower in 1984 than in any of the fertilizerd years (1980–83) (Fig. 3; Table 2). Seasonal chlorophyll *a* maxima occurred during spring in 1984 (2.30 $\mu g \cdot L^{-1}$) and during early fall in fertilized years (range = 2.65–3.43 $\mu g \cdot L^{-1}$) (Fig. 2). Although the abundance of algae decreased in 1984, algal mean volume was higher, suggesting a shift in phytoplankton species composition linked to nutrient supply (Fig. 3). Further evidence of change in the pelagic community was indicated by a greater compensation depth throughout the growing season in 1984 (Fig. 2).

During years of fertilization (1980–83), picoplankton (<3 μm) was clearly the most abundant size category of phytoplankton (Fig. 4). Cyanobacteria comprised up to 90% of the picoplankton in these years. Nanoplankton (3–20 μm) was the second most abundant group during 1981–83 with the exception of fall 1983, when microplankton (>20 μm) were observed in greater numbers due to increases in the abundance of *Melosira* spp., *Asterionella formosa, Tabellaria* spp., and *Mougeotia* spp. (Fig. 5). In the unfertilized year, numbers of picoplankton were lower than in previous years for most of the season. Microplankton was the dominant algal group by volume throughout the study, and a large early summer increase in microplankton was observed when nutrients were not added.

Cyanobacteria, mostly *Synechococcus* spp., was the most abundant taxonomic group in all years (1981–84) but was less abundant in the unfertilized condition. Chrysophytes, notably *Chromulina* spp., showed the opposite trend and were more numerous in 1984 than during fertilized years. The diatoms *Rhizosolenia* spp. (in early summer) and *Melosira* spp. (in fall) were more abundant with twice weekly treatment in 1983 than in previous years and increased again to even greater average densities in 1984. Diatoms or other large chrysophytes represented the greatest contribution to total phytoplankton volume in Hobiton Lake in all years of study. Dominant phytoplankton in spring were usually *Chromulina* spp. and *Rhizosolenia* spp., followed by *Aphanocapsa* spp., *Synechococcus* spp., and microcolonial cyanobacteria in the summer, and *Tabellaria* spp., *Mougeotia* spp., *Asterionella* sp., *Gymnodinium* spp., *Peridinium* spp., and *Melosira* spp. in the fall.

Zooplankton and Fish

Zooplankton abundance was inversely related to chlorophyll *a* concentration. Average seasonal density of zooplankton was lowest in 1981, the year of highest average chlorophyll *a* concentration, whereas the density of zooplankton was greatest in 1982, a year of low chlorophyll *a* concentration (Fig. 3 and 6). Numbers of the four most abundant zooplankton genera (*Eubosmina, Cyclops, Diaptomus,* and *Diaphanosoma*) were also highest in 1982 (Fig. 6). Average densities of medium

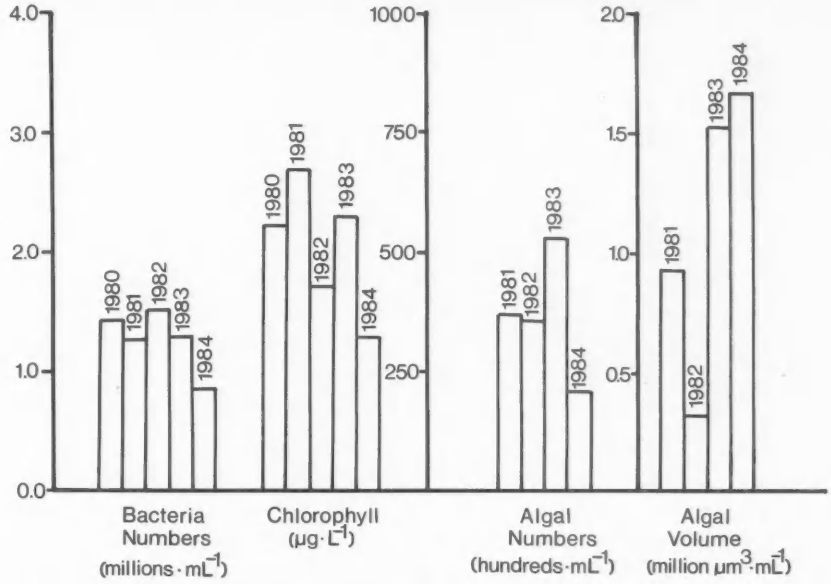

FIG. 3. Variability in growing season averages of bacteria numbers, chlorophyll concentration, algal numbers, and algal volumes for fertilized (1980–83) and unfertilized (1984) years.

TABLE 2. Seasonal averages of salient biological variables for Hobiton Lake, 1980–84.

Year		Bacteria (millions·mL^{-1})	Chlorophyll (μg·L^{-1})	Picoplankton (hundreds·mL^{-1})	Nanoplankton (hundreds·mL^{-1})	Microplankton (hundreds·mL^{-1})	Zooplankton (no.·m^{-3})	Juvenile sockeye (no.·ha^{-1})
1980	Mean	1.41	2.22	—	29.9	5.4	—	—
	Minimum	0.64	0.77	—	6.8	1.9	—	—
	Maximum	2.10	3.23	—	48.4	13.5	—	—
1981	Mean	1.25	2.69	329.8	27.7	6.6	2 388	1500
	Minimum	0.69	1.67	29.9	5.3	0.8	692	—
	Maximum	2.14	3.43	524.2	59.0	23.4	5 573	—
1982	Mean	1.53	1.73	248.8	77.6	29.2	5 228	1555
	Minimum	0.78	0.33	19.6	2.9	0.9	1 413	—
	Maximum	2.42	2.65	518.3	314.1	147.9	13 261	—
1983	Mean	1.26	2.30	346.6	133.0	103.5	3 597	1862
	Minimum	1.01	1.34	63.2	29.4	6.0	1 914	—
	Maximum	1.57	3.36	787.6	276.5	386.7	4 914	—
1984	Mean	0.84	1.30	93.2	99.1	39.0	4 329	1576
	Minimum	0.74	0.47	24.4	22.8	13.8	393	—
	Maximum	1.34	2.30	198.4	234.4	73.7	8 258	—

zooplankton (0.15–0.84 mm) primarily *Diacyclops bicuspidatus*, *Eubosmina coregoni*, and *Diaphanosoma* sp., and large zooplankton (0.85–1.5 mm), primarily *Cyclops vernalis* and *Diaptomus oregonensis*, were greatest in 1982 (3080 and 520·m^{-3}, respectively). However, rotifers and small zooplankton (0.1–0.14 mm) were most dense in 1984 (2345·m^{-3}) when nutrients were not added to Hobiton Lake. Densities of zooplankton were highest in 1982 despite similar estimates of juvenile sockeye salmon density (1500–1576 fish·ha^{-1}) for the years 1981, 1982, and 1984 (K. Hyatt, Pacific Biological Station, Nanaimo, B.C., pers. comm.) (Table 2).

Maximum densities of zooplankton occurred in either May (5570·m^{-3}, 1981; 13 260·m^{-3}, 1982), June (8260·m^{-3}, 1984), or July (4910·m^{-3}, 1983), with lower densities observed in spring and fall. All zooplankton species were at low densities in early spring, then usually *D. oregonensis* and rotifers reached maximum densities in April or May, followed by *E. coregoni* which reached maximum densities from May through July, and then *Diaphanosoma* sp. which peaked from June through August. *Cyclops* spp. reached maximum densities in different months each year.

Discussion

Seasonality, species composition, and abundance of phytoplankton communities in fast-flushing monomictic lakes in British Columbia were initially described by Stockner et al. (1980) and Shortreed and Stockner (1981), including a comparison of phytoplankton communities in Kennedy Lake before and during the first year of fertilization. In two recent studies,

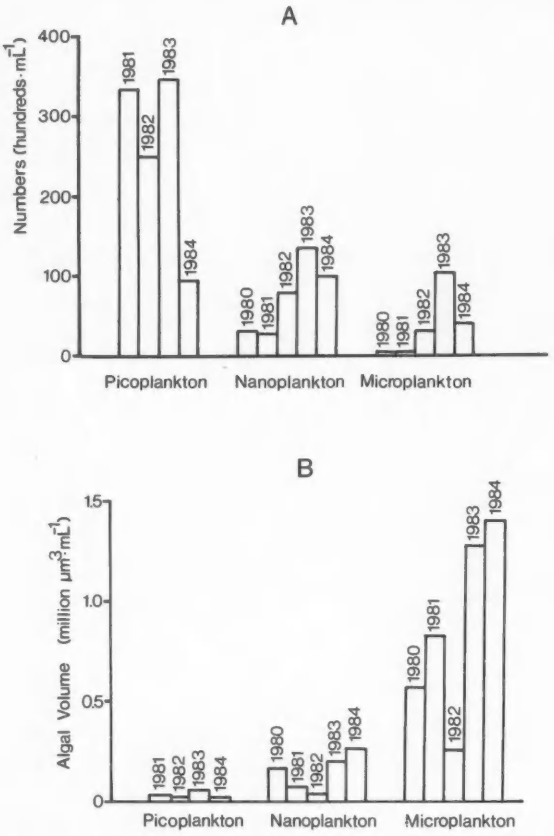

FIG. 4. Variation in seasonal averages of pico-, nano-, and microplankton (A) numbers and (B) volumes for fertilized (1980–83) and unfertilized (1984) years.

the effects of heavy metal contamination on the phytoplankton of Buttle Lake on Vancouver Island were reported (Austin and Muteanu 1984; Roch et al. 1985), and the effects of fertilization on algal species in Mohun Lake were described (Perrin et al. 1984). However, the most detailed description of a phytoplankton community in a coastal lake to date is by MacIsaac and Stockner (1985) who discussed the potential impact of coal mining on the plankton of Quinsam Lake, British Columbia. This study of Hobiton Lake presents a more comprehensive picture of the plankton communities of a typical fast-flushing coastal lake.

The relationship between bacteria numbers and total phosphorus has been reported for dimictic lakes of the Laurentian Shield in eastern Canada (Bird and Kalff 1984) and for subarctic lakes in the Yukon Territory (Shortreed and Stockner 1986). Both studies demonstrate the strong correlation between bacteria numbers and trophic state, and for comparative purposes, we have plotted data from Hobiton Lake with data from these studies (Fig. 7). While Hobiton Lake data are generally lower than data from either of the other studies, they are not outliers in the regression, which further illustrates the utility of bacteria as indicators of trophic status even in lakes as widely disparate as warm monomictic lakes of British Columbia and subarctic dimictic lakes in the Yukon Territory. The strong relationship between total phosphorus and bacteria numbers has also been observed in other British Columbia coastal lakes (E. MacIsaac, West Vancouver Laboratory, West Vancouver, B.C., pers. comm.). Based on a trophic scale defined by Bird and Kalff (1984) using bacteria numbers, Hobiton Lake lies within the oligotrophic category ($<1.7 \times 10^6$ bacteria·mL^{-1}).

The highest abundance of all size categories of phytoplankton and highest cell volume of microplankton (>20 μm) occurred in 1983 when the frequency of fertilization was increased from once to twice weekly. The most notable increases occurred in microplankton species such as the diatoms *Rhizosolenia* and *Melosira* and the filamentous chlorophyte *Mougeotia*. However, in 1983, zooplankton were not as abundant as in the previous year, most likely a result of a higher fish density (K. Hyatt, pers. comm.); thus, the greater densities of phytoplankton may be partially attributed not only to increased frequency of fertilization but also to reduced grazing rates.

After nutrient reduction in 1984, several chrysophyte species (*Asterionella formosa, Rhizosolenia* spp., *Melosira* spp., and *Chromulina* spp.) increased in abundance, resulting in a higher average cell volume in the unfertilized year. However, since *Rhizosolenia* and other large vacuolated diatoms have a low cellular carbon content (Stockner and Shortreed 1985), the use of cell volume may result in an overestimate of the contribution of microplankton and an underestimate of the contribution of picoplankton to total phytoplankton biomass. In contrast, following nutrient reduction in a eutrophic subarctic lake in northern Sweden, *Chromulina* spp. and other chrysophytes as well as chlorophytes decreased (Holmgren 1985). While changes in nutrient supply often produce predictable changes in phytoplankton biomass (Schindler 1977; Vollenweider 1976; Stockner and Shortreed 1985), numerous broad environmental variables such as solar radiation, temperature regime, and zooplankton grazing affect patterns of assemblage dominance and make changes in phytoplankton species composition difficult to predict on the basis of nutrients alone (Reynolds 1984).

During years of fertilization, average TN:TP ratios ranged from 38:1 to 52:1, considerably lower than the ratio of 129:1 which occurred in the unfertilized year. Smith (1983) demonstrated that lower TN:TP ratios favor dominance by cyanobacteria, and in the fertilized years, cyanobacteria (primarily *Synechococcus* spp.) were the most abundant phytoplankton in Hobiton Lake. A shift from small cyanobacteria to larger microplankton (especially *Rhizosolenia* spp.) occurred in 1984 when the average TN:TP ratio was higher. Increases in the abundance of *Rhizosolenia* spp. also occurred following increased dissolved nitrogen loads to Mohun Lake, B.C. (Perrin et al. 1984). In another British Columbia coastal lake (Nimpkish), *Rhizosolenia* spp. became dominant when fertilizer was added in an N:P ratio of 15:1 (1982) and then were far less abundant the following year when the N:P ratio was lowered to 1:1 (unpublished data). In the laboratory, a diatom (*Asterionella formosa*) has been shown to dominate over a colonial cyanobacteria (*Microcystis aeruginosa*) under phosphorus limitation (Holm and Armstrong 1981), and growth rates of *Rhizosolenia* spp. in laboratory cultures are higher with a high N:P ratio (E. MacIsaac, pers. comm.). These data suggest that the increase in abundance of *Rhizosolenia* spp. in the unfertilized year in Hobiton Lake was partially due to changes in N:P ratios.

Smith and Kalff (1982) showed that the sensitivity of growth rate to cell size decreases with increasing cell size and that the

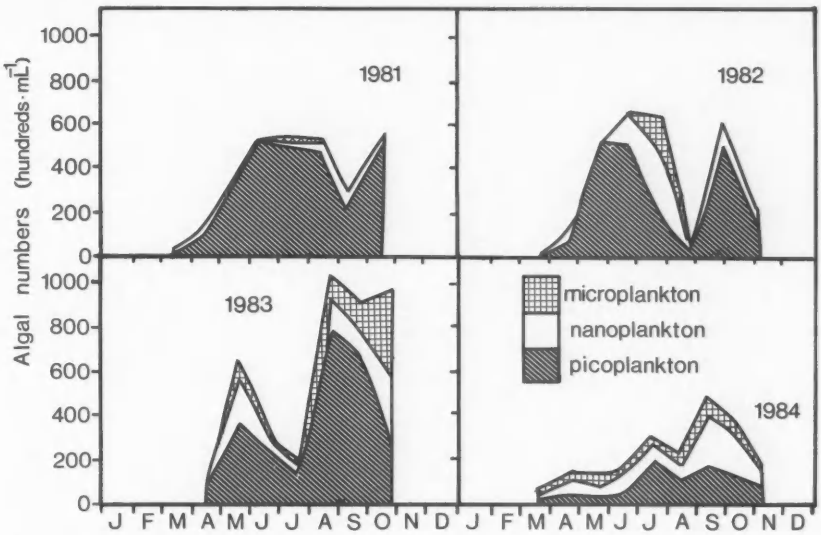

FIG. 5. Seasonal variation in pico-, nano-, and microplankton numbers for fertilized (1981–83) and unfertilized (1984) years.

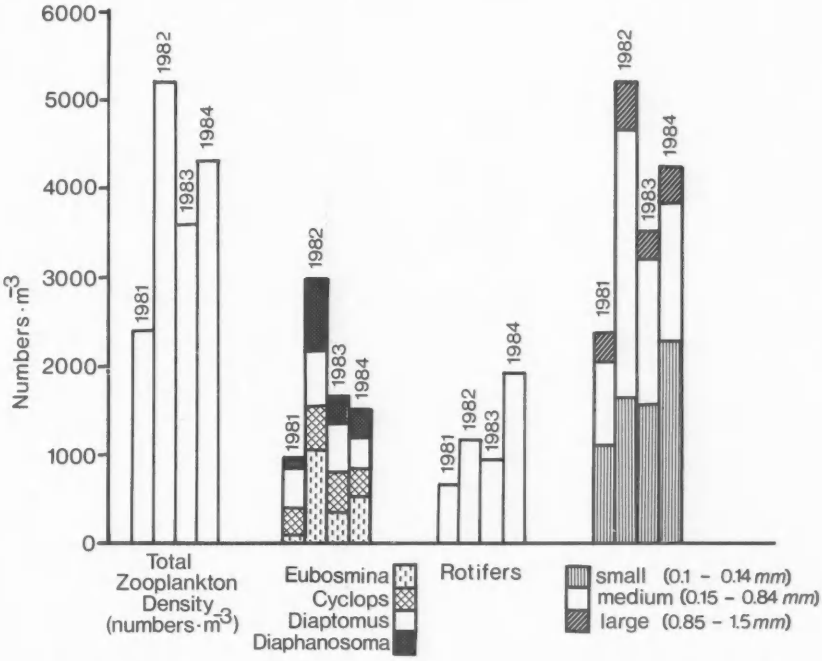

FIG. 6. Comparison of growing season average numbers of total zooplankton, of the four most abundant zooplankton genera, of rotifers, and of small, medium, and large zooplankton in Hobiton Lake, 1981–84.

differential in growth rates due to size decreases as intensity of competition increases. They predicted that the better nutrient competitors (smaller cells) are at less of an advantage when competition is severe or moderate (as in the unfertilized year) that when it is slight (as in fertilized years, 1980–83). This prediction fits observations from Hobiton Lake; smaller cells were more abundant during years of fertilization (1980–83). The fertilization of three subarctic lakes in northern Sweden also favored small species (Holmgren 1984); dense growths of chlorococcoid picoplankton occurred following nutrient enrichment. Observations from Hobiton Lake and the Swedish lakes are in contrast with the suggestion by Watson and Kalff

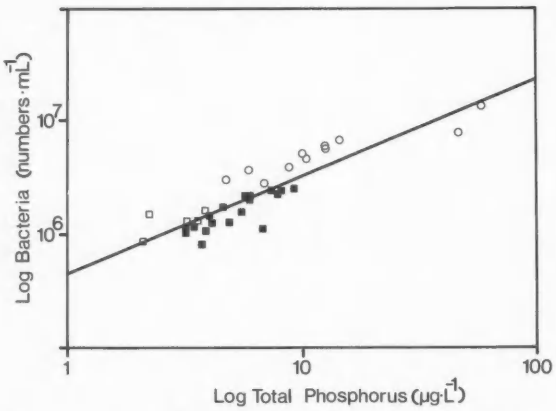

FIG. 7. Log–log plot of bacteria numbers and total phosphorus for a warm, monomictic lake (Hobiton Lake) (□), subarctic Yukon lakes (■), and dimictic lakes in eastern Canada (○) ($r^2 = 0.81$, $n = 35$, $p < 0.01$).

(1981) that fertilization and pollution (i.e. increased trophy) result in a decreased relative proportion of nanoplankton.

Phototrophic picoplankton (<3 μm) in Hobiton Lake comprised up to 90% (1981) of total phytoplankton numbers, up to 5% of total phytoplankton biomass (1982), and were likely major contributors to primary production. Costella et al. (1979) showed that on an annual basis, phototrophic picoplankton (<3 μm), while contributing only 6% to chlorophyll biomass in Great Central Lake, B.C., accounted for up to 20% of carbon production. Similarly, Stockner and Antia (1986) have shown in several British Columbia coastal lakes that picoplankton contributed from 20 to 70% of total daily and annual production while representing only 5–20% of chlorophyll biomass. Picoplankton have been found to be a significant part of the biomass in New Zealand lakes of mixed trophic status where about 30% of the biomass of plankton was <3 μm in size (Paerl 1977). Chroococcoid cyanobacteria constituted 30% of the biomass of all microorganisms <20 μm in size in Lake Ontario (Caron et al. 1985), up to 50% of both biomass and production in Lake Superior (Fahnenstiel et al. 1986), and up to 50% of the algal biomass (in autumn) in a small meromictic lake in Ontario (Craig 1983). Other studies (Berman 1975; Johnson and Sieburth 1979; Larsson and Hagstrom 1982; Li et al. 1983; Platt et al. 1983) have demonstrated that in the oceans, autotrophic picoplankton contribute significantly to total phytoplankton biomass and production.

Nutrient loading appears to be of greater importance than zooplankton grazing in determining phytoplankton size–frequency in Hobiton Lake; high planktivorous fish densities in coastal British Columbia lakes keep zooplankton populations and thus grazing pressure relatively low (Hyatt and Stockner 1985; Stockner and Shortreed 1985). One change in the zooplankton community following nutrient reduction was an increased abundance of rotifers relative to the previous years, an observation in contrast with several reports of increased rotifer abundance with nutrient addition (Krogius and Krokhin 1948; Nelson and Edmondson 1955; Donaldson et al. 1971). In coastal British Columbia lakes, a shift to rotifers and small zooplankton has been observed in the presence of increased grazing pressure by fish on the larger zooplankton species (K. Hyatt, pers. comm.). In the oligotrophic ocean, a large percentage (95%) of daily grazing is by herbivorous microzooplankton (Conover 1982; Welschmeyer and Lorenzen 1985). The significance of microzooplankton grazing in coastal British Columbia lakes, especially those with high grazing pressure by fish, may be of greater importance than previously realized.

De Bernardi et al. (1981) reported that cyanobacteria can be efficiently utilized by filter-feeding macrozooplankton if the colonies are small and that low utilization of cyanobacteria by filter-feeding zooplankton is attributable to the size of their colonies rather than their indigestibility. *Synechococcus* spp., the primary component of picoplankton in Hobiton Lake, are single celled with average cell dimensions of 0.5–2 μm; therefore, they are theoretically available for use by both micro- and macrozooplankton. Although heterotrophic flagellates or ciliates were not enumerated in this investigation, their occurrence in British Columbia coastal lakes has been confirmed (Stockner 1986; F. Hardy, unpubl. data). It is now commonly acknowledged that the protozoan component of microzooplankton communities in lakes and oceans is an exceedingly active metabolic unit, grazing on bacteria and algal picoplankton and serving as prey for microzooplankton (Williams 1981; Fenchel 1982; Johnson and Sieberth 1982; Azam et al. 1983; Porter et al. 1985). Further investigation is needed to elucidate the pathways and efficiency of energy transfer from picoplankton to macrozooplankton to fish, or from picoplankton to microzooplankton to macrozooplankton to fish, following nutrient addition in British Columbia coastal lakes.

Nutrient reduction in Hobiton Lake resulted in decreased total numbers of bacteria and phytoplankton, especially picoplankton. Phytoplankton volume was highest in the unfertilized year as a result of increases in several large diatom species. Chlorophyll *a* concentrations were lower in the unfertilized year, particularly in late summer and early autumn, than in the years of fertilization. We believe that availability of food for zooplankton during this period is a critical factor in the success of fertilization as a sockeye salmon enhancement technique, and differences during this period may explain why sockeye fry survival was substantially lower in 1984 than in the previous three years (K. Hyatt, pers. comm.). Since the greatest changes in phytoplankton numbers after nutrient reduction occurred in the picoplankton, it is imperative to determine the importance of this size category as a food source of the zooplankton community.

Acknowledgments

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Loss of Lampricides by Adsorption on Bottom Sediments

V. K. Dawson

U.S. Fish and Wildlife Service, National Fishery Research Laboratory, P.O. Box 818, La Crosse, WI 54602-0818, USA

D. A. Johnson

U.S. Fish and Wildlife Service, Marquette Biological Station, 446 E. Crescent Street, Marquette, MI 49855, USA

and J. L. Allen

U.S. Fish and Wildlife Service, National Fishery Research Laboratory, P.O. Box 818, La Crosse, WI 54602-0818, USA

Dawson, V. K., D. A. Johnson, and J. L. Allen. 1986. Loss of lampricides by adsorption on bottom sediments. Can. J. Fish. Aquat. Sci. 43: 1515–1520.

Problems have been encountered in maintaining effective concentrations of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 5,2′-dichloro-4′-nitrosalicylanilide (Bayer 73) during treatments of certain Great Lakes tributaries. Concentrations of Bayer 73 decreased by more than 80% in a portion of the Ford River, Michigan, during treatment in 1980. Adsorption of Bayer 73 on sediments was hypothesized as the primary mechanism of this excessive loss. Subsequent laboratory studies demonstrated that lampricides are adsorbed by silt-type sediments that are high in organic content, including those collected from the treated portion of the Ford River. Un-ionized lampricides (acidic solution) were more readily adsorbed than ionized forms (basic solution). Adsorption onto sediments was proportionally greater, and desorption proportionally less, for Bayer 73 than for TFM. When Ford River sediments were mixed with lampricide-free water, less than 10% of the adsorbed Bayer 73, but more than 60% of the TFM, was released. The extensive adsorption of the lampricides (especially Bayer 73) from solution by silt-type sediments explains much of the loss of effective concentrations in certain streams.

Le maintien de concentrations efficaces de lamprocides a posé des difficultés au cours du traitement de certains tributaires des Grands Lacs. Les produits en cause sont le 3-trifluorméthyl-4-nitrophénol (TFM) et le 5,2′-dichloro-4′-nitrosalicylanilide (Bayer 73). Les concentrations de ce dernier ont chuté de plus de 80 % dans une partie de la rivière Ford au Michigan pendant le traitement en 1980. On a formulé l'hypothèse d'adsorption du Bayer 73 sur les sédiments comme mécanisme primaire de cette perte excessive. Par la suite, des études de laboratoire ont montré que les lamprocides sont adsorbés par des sédiments vaseux à forte teneur organique, y compris ceux prélevés de la partie traitée de la rivière Ford. Les lamprocides non ionisés (solution acide) sont plus rapidement adsorbés que les formes ionisées (solution alcaline). L'adsorption sédimentaire était proportionnellement plus forte et la désorption proportionnellement plus faible pour le Bayer 73 que pour le TFM. Lorsque des sédiments de la rivière Ford furent mélangés à de l'eau sans lamprocide, moins de 10 % du Bayer 73 mais plus de 60 % du TFM furent libérés. La forte adsorption des lamprocides en solution (particulièrement du Bayer 73) par les vases sédimentaires explique en grande partie la perte de concentrations efficaces dans certains cours d'eau.

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The sea lamprey (*Petromyzon marinus*) has substantially contributed to extinction of lake trout (*Salvelinus namaycush*) in Lakes Huron and Michigan and the reduction of the population in Lake Superior to record low levels (Applegate et al. 1961). The chemical 3-trifluoromethyl-4-nitrophenol (TFM) was found to be selectively toxic to sea lamprey larvae, and methodology for its application to streams was developed (Applegate et al. 1961). It was later discovered that the addition of small amounts of 5,2′-dichloro-4′-nitrosalicylanilide (Bayer 73 or Bayluscide) reduced the concentration of TFM needed to kill sea lamprey larvae without significantly affecting its selectivity (Howell et al. 1964).

Occasionally, treatments involving the use of Bayer 73 have not been as effective as predicted by pretreatment toxicity tests (Howell and Marquette 1962). An example of a treatment failure involving an application of Bayer 73 occurred on the Ford River in the Upper Peninsula of Michigan in 1971. Post-treatment surveys of ammocoete survival indicated that the treatment was not effective. The cause of the failure was not known, but photodegradation of Bayer 73 was suspected (unpublished treatment report). In 1974, the Ford River was again treated with a mixture of TFM and Bayer 73. The percentage of Bayer 73 was doubled to allow for the suspected loss. This time, the treatment was successful.

A gas–liquid chromatographic method of analyzing for Bayer 73 was later developed (Luhning et al. 1979) and incor-

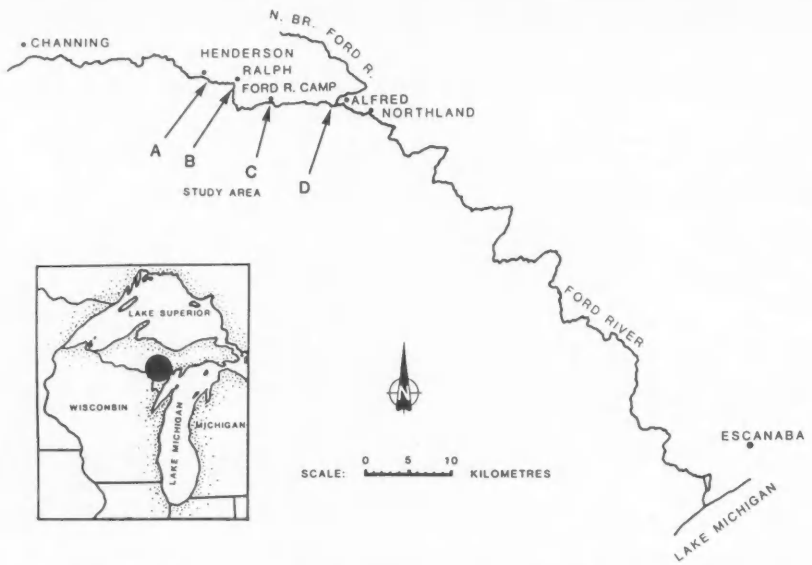

FIG. 1. Water sample monitoring sites (A–D) during lampricide treatment of the Ford River, Michigan.

TABLE 1. Characteristics of water samples from three sites on the Ford River on June 11, 1980, just before lampricide treatment.

Sites[a]	Flow (m^3/s)	Temperature (°C)	Alkalinity (mg/L as $CaCO_3$)	Conductivity mS/m)	pH
Henderson 0.4 km downstream)	1.22	12	112	25	8.1
Ralph	1.22	10	134	25	8.0
Alfred	2.24	12	121	25	8.0

[a]See Fig. 1 for locations of sites.

porated into routine field operations in 1979. The first opportunity to use this method to monitor Bayer 73 concentrations in the Ford River came in 1980.

We describe the 1980 treatment of the Ford River and the subsequent laboratory studies performed to determine if adsorption by certain sediments was contributing to the loss of lampricides during stream treatments.

Materials and Methods

Chemicals

A liquid formulation of TFM (35.9% active ingredient) manufactured by American Hoechst Corporation was applied. The Bayer 73 used was a 70% active wettable powder obtained from the Chemagro Agricultural Division of Mobay Chemical Company. Because of its low solubility in water, the 70% wettable powder was applied in a water slurry. The slurry was metered into a sump, where it was mixed with TFM and stream water. It was then applied by pumping the mixture through a spreader hose strung across the stream.

Ford River Treatment

Treatment of the Ford River began on May 31, 1980, with an application of TFM at Channing, Michigan (Fig. 1). Additional TFM in combination with Bayer 73 was added at Henderson on June 2. The lampricides were expected to remain lethal to sea lampreys downstream to Alfred. However, when analyses indicated that the concentrations of both lampricides had dropped below the minimum lethal concentration (Kanayama 1963), the section of the stream from Henderson to Alfred was treated again.

The second application of TFM and Bayer 73 at Henderson began on June 12. TFM was applied for 12 h at 7.0 mg/L, and 0.072 mg/L of Bayer 73 was applied to ensure that a lethal concentration would extend downstream to Alfred. Water samples were collected at a site 0.4 km downstream from Henderson, at Ralph, at Ford River Camp, and just upstream from the confluence of the Ford River and its North Branch in order to closely monitor residues of the lampricides during the second application. TFM was analyzed by colorimetry (Smith et al. 1960) and Bayer 73 by gas–liquid chromatography (Luhning et al. 1979). Water samples were collected by hand, except at Ford River Camp where an ISCO® automatic sampler was used.[1] Selected physical and chemical data were collected at three sampling points before the lampricide was applied (Table 1).

[1]Use of trade names does not imply U.S. Government endorsement of commercial products.

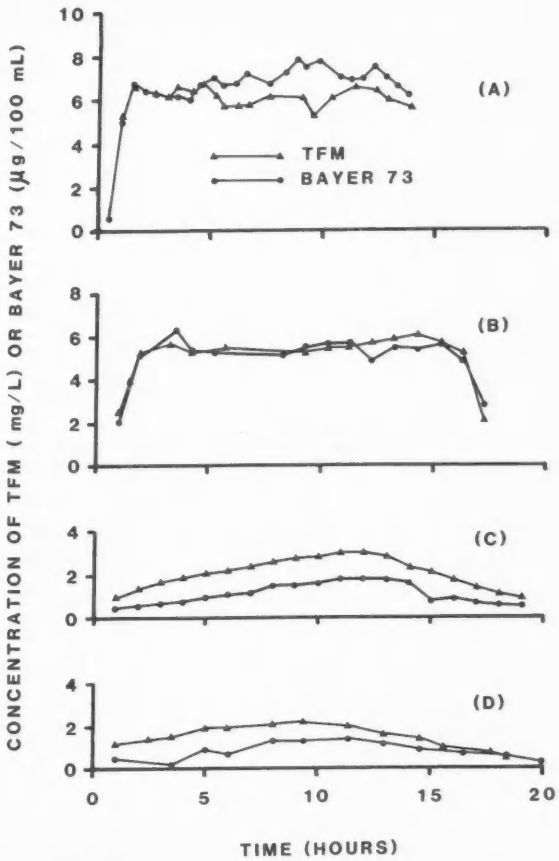

FIG. 2. Concentrations of TFM and Bayer 73 in water samples collected 0.4 km downstream from (A) Henderson beginning at 8:30 a.m. June 13, (B) at Ralph beginning at 1:00 p.m. June 13, (C) at Ford River Camp beginning at 3:00 a.m. June 14, and (D) at Alfred beginning at 4:00 p.m. June 14 during the lampricide treatment of the Ford River, Michigan.

Laboratory Adsorption Studies

In a preliminary study of adsorption of Bayer 73, we used bottom sediment samples collected at Henderson, Michigan, 50 m upstream from the Bayer 73 application point. Two types of substrate were collected: black mud, rich in organic detritus, was taken from an area of low water velocity near the shore; and a silt–sand mixture, also rich in detritus but more coarse in texture, was collected from the center of the stream. Excess water in the samples was drained through Whatman 2V® filter paper (5–10 µm retention). The sediment samples (50 mL) were shaken for 1 min in separatory funnels containing 500 mL of Ford River water spiked with 60 µg Bayer 73/L. A spiked control without sediment was also carried through the procedure. The mixtures were sampled before and after filtration through filter paper and analyzed by gas–liquid chromatography. Because significant amounts of Bayer 73 were adsorbed by the filter paper, centrifugation was used for subsequent separations.

Sediments from the Cedar, Tahquamenon, and Ford rivers in the Upper Peninsula of Michigan were selected for the detailed study of their effects on lampricides because they represented a wide range of sediment types. These were analyzed for particle size, using standardized procedures for sieving and hydrometer classification (American Society for Testing and Materials 1979). Adsorptive and desorptive properties were evaluated against ^{14}C-labeled TFM (specific activity 135 GBq/mol) and Bayer 73 (specific activity 370 GBq/mol).

TABLE 2. Concentrations of Bayer 73 (µg/L) in stream water before and after laboratory exposure to sediment from the Ford River.

Medium	Before exposure (µg/L)[a]	Postexposure			
		Unfiltered		Filtered	
		µg/L	% loss	µg/L	% loss
Black mud	57	6.5	89	<2.0	>96
Silt–sand	61	31	49	8.5	86
Control	57	60	—	49	14

[a]Each value is the mean for two tests.

In conducting adsorption tests, we used a series of lampricide concentrations (0.1, 0.2, 0.4, 0.6, and 1.0 mg/L), a blank containing sediment, a blank without sediment, and a control containing lampricide (0.5 mg/L) without sediment. We mixed 20 mL of ^{14}C-labeled TFM or ^{14}C-labeled Bayer 73 solutions with 2.0 g (dry weight) of sediment in 125-mL screw-capped Erlenmeyer flasks, and then agitated the mixtures on a Lab Line® model 3590 orbital shaker at 100 RPM for at least 16 h to attain adsorption equilibria. Temperature (20 ± 1°C) was controlled by an environmental chamber, and pH's were maintained by using phosphate or carbonate buffers (0.1 *M*). After equilibrium was reached, the mixtures were transferred to glass centrifuge tubes and centrifuged on an International model UV centrifuge at 1000 × *g* for 30 min. Sample temperature was maintained at 20°C throughout the process. After centrifugation, duplicate 250-µL samples of the liquid phases were transferred by micropipette to scintillation vials containing 10 mL of Beckman Ready-solv® HP scintillation cocktail. The samples were counted on a Beckman model 7500 scintillation counter programmed to correct for quench, background, and counting efficiency. An additional 15.5 mL of liquid was removed from each centrifuge tube, and then 16 mL of buffered water was used to rinse the sediment back into the Erlenmeyer flask and bring the total volume of liquid back to 20 mL. The flasks were again placed on the orbital shaker to equilibrate (>16 h), centrifuged, and then sampled as above for scintillation counting. The resulting radioactivity was corrected for the amount of ^{14}C-labeled lampricide remaining in the 4 mL of liquid retained with the sediment to determine the amount of chemical that desorbed from the sediments.

Results and Discussion

Ford River Treatment

The lampricides were applied at Henderson and water samples were monitored for residues of the chemicals at four sites in the study area (Fig. 1). At the first site (0.4 km downstream from Henderson), the lampricide block was 12 h long (i.e. it required 12 h to pass a given point), and the mean Bayer 73 concentration was 0.066 mg/L (Fig. 2A). At Ralph, where the block was about 14 h long, the mean Bayer concentration had dropped to 0.054 mg/L (Fig. 2B). At the Ford River Camp, the

TABLE 3. Characteristics of sediments used to evaluate adsorptivity.

Characteristic	River		
	Tahquamenon	Ford	Cedar
Soil type	Sand	Loamy sand	Sandy loam
% sand	96	84	64
% silt	2	14	32
% clay	2	2	4
Calcium (μg/g)	200	800	3000
Magnesium (μg/g)	50	250	300
Potassium (μg/g)	25	20	43
Phosphorus (μg/g)	13	13	9.5
Cation exchange capacity (meq/100 g)	1.1	4.6	13.2
% organic	0.9	5.0	9.0
pH	6.5	6.3	6.8

TABLE 4. Average (±SE, $n = 5$) sorption coefficients (K_d) for TFM and Bayer 73 on bottom sediments at selected pH's and 20°C.

Chemical and sediment source	pH			
	6[a]	7	8	9
TFM				
Cedar River	11.7±0.682	5.99±0.040	2.01±0.167	1.93±0.219
Ford River	6.65±0.246	3.58±0.262	1.46±0.091	1.06±0.108
Tahquamenon River	1.11±0.016	0.316±0.013	0.157±0.012	0
Bayer 73				
Cedar River	316±15.2	85.1±14.8	68.9±6.74	7.01±0.262
Ford River	60.3±5.63	78.7±3.32	41.4±3.04	11.7±1.63
Tahquamenon River	17.3±0.673	14.2±1.43	4.79±0.386	1.18±0.128

[a]Due to solubility problems, pH 6.5 was used for Bayer 73.

TABLE 5. Sorption constants (K_{oc}) for TFM and Bayer 73 on bottom sediments at selected pH's and 20°C.

Chemical and sediment source	pH			
	6[a]	7	8	9
TFM				
Cedar River	130	66.6	22.3	21.4
Ford River	133	71.6	29.2	21.2
Tahquamenon River	123	35.1	17.4	0
Bayer 73				
Cedar River	3510	946	766	77.9
Ford River	1210	1570	828	234
Tahquamenon River	1920	1580	532	131

[a]Due to solubility problems, pH 6.5 was used for Bayer 73.

length of the lampricide block had increased to more than 18 h, and the mean Bayer 73 concentration during the 12 h when lampricide concentrations were highest was reduced to 0.013 mg/L (Fig. 2C). At Alfred, Bayer 73 was present for 19 h; mean concentration during the 12 h when it was highest was only 0.011 mg/L (Fig. 2D). Concentrations of TFM also were reduced, but not as sharply as those of Bayer 73. In spite of the reduced lampricide concentrations, posttreatment surveys indicated that the second treatment was effective (Dale Ollila, Marquette Biological Station, Marquette, MI, pers. comm.). Dilution and lengthening of the lampricide block by stream hydraulics accounted for some of the loss of the two chemicals in the study area. However, the amount of Bayer 73 lost was unexpectedly high, especially between Henderson and Ford River Camp (Fig. 2). At Henderson, Bayer 73 concentrations averaged 1.12% of the TFM concentrations. At Ralph, Bayer 73 had dropped to 0.99%, and at Ford River Camp and Alfred, Bayer 73 was only 0.55% of the TFM concentration.

Although both lampricides are degraded by photolysis (Dawson 1973; Schultz and Harman 1978; Carey and Fox 1981), it is unlikely that photolytic degradation of Bayer 73 was extensive; during the treatment, most of the lampricide block passed between Ralph and Ford River Camp after sunset. Furthermore, the loss of Bayer 73 could not be explained by hydrolysis because little or no hydrolysis of Bayer 73 in aqueous solutions has been demonstrated (Schultz and Harman 1978). The Ford River is shallow and has fine, organic sediments in the area between Ralph and Ford River Camp; consequently the lampricides were exposed to a large surface area of substrate, and adsorption seems the more likely mechanism of Bayer 73 loss.

Laboratory Adsorption Studies

In the preliminary adsorption study, sediments from the Ford River removed Bayer 73 from fortified river water samples (Table 2). Concentrations of Bayer 73 in water samples exposed to black mud were reduced by 89% before filtration; only trace amounts remained after filtration (detection limit <2.0 μg/L). The loss from the solution exposed to the silt–sand sediment was 49%, and an additional 37% was lost during filtration. The procedural control lost no Bayer 73 to the glass separatory funnel, but the concentration was reduced 14%

TABLE 6. Average (±SE, $n = 3$) sorption constants (K_{oc}) for TFM and Bayer 73 on bottom sediments at selected pH's and corresponding mobility class for the chemicals in soil.

Chemical and sorption characteristic	pH			
	6[a]	7	8	9
TFM				
K_{oc}	129±2.96	57.8±11.4	23.0±3.42	14.2±7.10
Mobility class	High	High	Very high	Very high
Bayer 73				
K_{oc}	2210±680	1360±210	709±90.1	148±45.8
Mobility class	Slight	Low	Low	Medium

[a]Due to solubility problems, pH 6.5 was used for Bayer 73.

TABLE 7. Estimated leaching distances (cm)[a] for TFM and Bayer 73 in bottom sediments at selected pH's and 20°C.

Chemical and sediment source	pH			
	6[b]	7	8	9
TFM				
Cedar River	2.5	5.8	19.2	20.1
Ford River	5.1	10.3	>25	>25
Tahquamenon River	>25	>25	>25	>25
Bayer 73				
Cedar River	0	0	0	4.8
Ford River	0	0	0	2.5
Tahquamenon River	1.3	1.9	7.5	>25

[a]Based on inverse relationship with sorption coefficient (K_d) (McCall et al. 1981).

[b]Due to solubility problems, pH 6.5 was used for Bayer 73.

by filtration through Whatman 2V® paper.

Sediment samples collected for study of the adsorption of radiolabeled lampricides were characterized by analysis of particle size and selected chemical constituents. The sediment sample from the Tahquamenon River was predominantly sand, with only 2% silt and 0.9% organic content; the sample from the Ford River was a loamy sand with 14% silt and 5% organic content; and the sample from the Cedar River was a sandy loam with 32% silt and 9% organic content (Table 3).

The equilibrium that develops between the amounts of chemical in solution and the amounts adsorbed to sediments can be represented by adsorption equilibria plots. Preliminary studies with both lampricides indicated that this equilibrium was reached within 16 h on an orbital shaker. Equilibration time for most chemicals is between 4 and 24 h (Hance 1967). Lampricides in the predominately un-ionized form (acidic solution) were adsorbed by sediments to a greater degree than those in the predominately ionized form (basic solution). The extremely low solubility and precipitation of Bayer 73 at pH's below 6.5 precluded use of pH 6.0 buffers. Therefore, the Bayer 73 data used for comparison with TFM at pH 6.0 were those collected at pH 6.5.

Sorption coefficients (K_d), which represent the amount of chemical adsorbed by sediments divided by the equilibrium concentration in solution, were used to summarize the adsorption data. The larger the sorption coefficient, the more chemical that is adsorbed from solution. Sorption coefficients for TFM and Bayer 73 with each of the three sediment types are presented in Table 4. There was a significant ($P \leq 0.05$) inverse correlation between sorption coefficient and pH. Coefficients ranged from 0 to >10 for TFM and from about 1 to >300 for Bayer 73 depending on the pH and sediment source. Regressions of sorption coefficients against sediment characteristics indicated that the best correlation ($r^2 = 0.948$ for TFM and 0.890 for Bayer 73) related to percent organic content.

Sorption coefficients were normalized for the organic content of the various sediments by dividing the K_d by percent organic content. This value times 100 yielded the sorption constants (K_{oc}) presented in Table 5. Average values of K_{oc} ranged from 14 to 129 for TFM and from 148 to 2210 for Bayer 73 depending on pH (Table 6). Swann et al. (1983) reported sorption constants for a variety of chemicals ranging from 60 for 2,4-D to 500 000 for 2,2′,4,5,5′-PCB.

Sorption constants (K_{oc}) are used to classify the expected mobility of a chemical in soil. According to the classification system presented by Swann et al. (1983), the mobility of TFM in soil would be high to very high and that of Bayer 73 in soil would be slight to medium. There is an inverse relationship between the sorption coefficient (K_d) and leaching distance in soils (McCall et al. 1981). The expected leaching distance in soils ranges from 2.5 to >25 cm for TFM and from 0 to >25 cm for Bayer 73 (Table 7). Leaching distance for both lampricides increases as the soil becomes more sandy (lower organic content) and as pH increases (ionizing the lampricide molecule).

After the lampricide solutions were replaced with lampricide-free buffered solution, we determined the degree of desorption from sediments. There was little desorption (<10%) of Bayer 73 from the Ford River or Cedar River sediments. By contrast, more than 60% of TFM adsorbed to these sediments was released into freshwater. With the sandy Tahquamenon River sediments, less than 30% of the Bayer 73 was desorbed, while TFM was completely desorbed.

The fact that certain sediments do not readily release adsorbed Bayer 73 helps explain the losses of effective concentrations occasionally encountered. Even in situations where the lampricides readily desorb from sediments in freshwater, the adsorption–desorption process probably contributes to the lengthening and tailing of the chemical block.

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Effects of Clear-cut Logging with and without Buffer Strips on Juvenile Salmonids in Alaskan Streams[1]

Michael L. Murphy, Jonathan Heifetz, Scott W. Johnson, K V. Koski, and John F. Thedinga

Northwest and Alaska Fisheries Center Auke Bay Laboratory, National Marine Fisheries Service, NOAA, P.O. Box 210155, Auke Bay, AK 99821, USA

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To assess short-term effects of logging on juvenile *Oncorhynchus kisutch*, *Salvelinus malma*, *Salmo gairdneri*, and *Salmo clarki* in southeastern Alaska, we compared fish density and habitat in summer and winter in 18 streams in old-growth forest and in clearcuts with and without buffer strips. Buffered reaches did not consistently differ from old-growth reaches; clear-cut reaches had more periphyton, lower channel stability, and less canopy, pool volume, large woody debris, and undercut banks than old-growth reaches. In summer, if areas had underlying limestone, clear-cut reaches and buffered reaches with open canopy had more periphyton, benthos, and coho salmon fry (age 0) than old-growth reaches. In winter, abundance of parr (age >0) depended on amount of debris. If debris was left in clear-cut reaches, or added in buffered reaches, coho salmon parr were abundant (10–22/100 m^2). If debris had been removed from clear-cut reaches, parr were scarce (<2/100 m^2). Thus, clear-cutting may increase fry abundance in summer in some streams by increasing primary production, but may reduce abundance of parr in winter if debris is removed. Use of buffer strips maintains or increases debris, protects habitat, allows increased primary production, and can increase abundance of fry and parr.

Afin d'évaluer les effets à courte échéance du déboisement sur les formes juvéniles d'*Oncorhynchus kisutch*, de *Salvelinus malma*, de *Salmo gairdneri* et de *Salmo clarki* du sud-est de l'Alaska, nous avons comparé la densité de population ainsi que l'habitat de ces poissons tant en été qu'en hiver dans 18 cours d'eau de régions boisées et de régions déboisées, avec et sans bandes-tampons. Les régions tamponnées n'ont pas été trouvées constamment différentes des régions boisées; par ailleurs, les régions déboisées possédaient plus de périphyton, un lit moins stable, un couvert de verdure moins fourni, des bassins de moindre volume, moins de gros branchages et de rives affouillées que les régions comparables dans les boisés. Pendant l'été, dans les régions à fonds calcaires, les surfaces tant déboisées que tamponnées avec couvert dégarni comportaient plus de périphyton, de benthos et d'alevins de saumon coho (âge 0) que les régions boisées. En hiver, l'abondance des tacons (âge >0) dépendait de la quantité de débris. Lorsque les parties droites des cours d'eau des régions déboisées comportaient des débris, ou s'il en avait été ajouté dans les régions tamponnées, les tacons de saumon coho abondaient (10–22/100 m^2). Lorsque les débris avaient été retirés des cours rectilignes dans les régions déboisées, les tacons se faisaient rares (<2/100 m^2). Le déboisement peut donc augmenter l'abondance des alevins en été dans certains cours d'eau en favorisant la production primaire mais il risque de diminuer l'abondance des tacons en hiver si les débris ont disparu. La création de bandes-tampons conserve ou augmente les débris, protège l'habitat, favorise la production primaire et peut augmenter l'abondance des alevins et des tacons.

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Southeastern Alaska has the most extensive old-growth forest remaining in the United States. About 1.4 million ha of this forest, mainly western hemlock (*Tsuga heterophylla*) and Sitka spruce (*Picea sitchensis*), is commercial quality available for harvest, and about 8100 ha (0.6%) is harvested annually (Lentfer et al. 1980). Clear-cutting is the most common method of timber harvest, and trees are usually cut to the stream bank. To protect fish habitat, trees are normally felled away from streams and suspended when yarded across streams. Strips of uncut, streamside trees (buffer strips) are seldom left to protect fish habitat because the exposed trees may blow down.

Commercial and sport fisheries are a major contribution to the region's economy. Southeastern Alaska annually produces about 20 million Pacific salmon (*Oncorhynchus* spp.), worth over $100 million. All five species of Pacific salmon occur in southeastern Alaska, as well as steelhead trout (*Salmo gairdneri*), cutthroat trout (*S. clarki*), and Dolly Varden (*Salvelinus malma*).

Logging can be either beneficial or detrimental to fish, depending on the extent of changes in habitat and the species or life stage affected. Juvenile salmonids can be more abundant in recently logged areas (<15 yr old) than in nearby forested areas. Increased light reaching the stream after canopy removal can stimulate aquatic primary and secondary production (Murphy and Hall 1981; Bisson and Sedell 1984). Fish growth may also be enhanced after logging by warmer stream temperature which accelerates emergence and extends the growing

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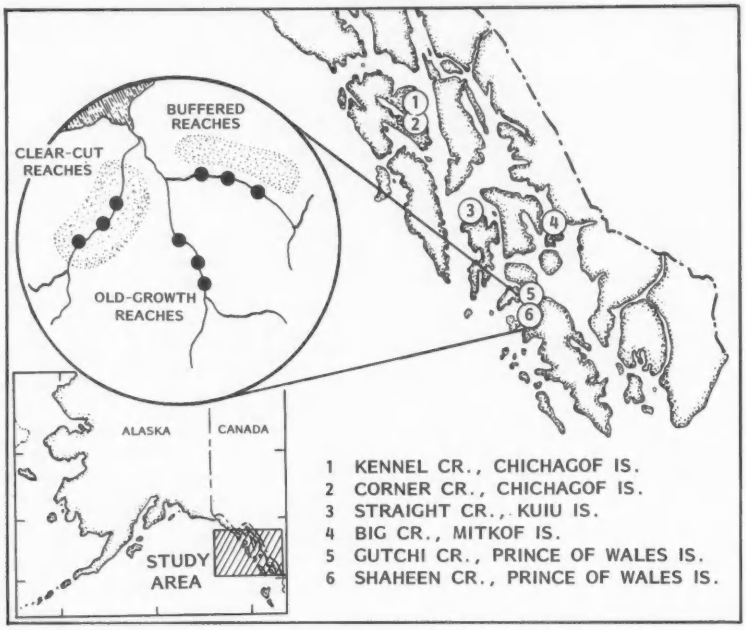

FIG. 1. Location of the study sites in southeastern Alaska and an example of a block, treatments, and study reaches (solid circles). The creeks listed are the major streams in each block (Big Creek is also informally known as Bear Creek). Treatment reaches were usually located on unnamed tributaries of these streams or in adjacent watersheds.

season (Holtby and Hartman 1982; Scrivener and Andersen 1984).

On the other hand, salmonid populations may decline after logging because of detrimental changes in physical habitat. These changes include excessive sedimentation (Platts and Megahan 1975; Scrivener and Brownlee 1982), less dissolved oxygen and elevated temperature (Ringler and Hall 1975), loss of large woody debris, collapsed stream banks, and decreased channel stability (Tschaplinski and Hartman 1983; Bisson and Sedell 1984). Features of fish habitat that need protection during logging must be identified so that timber can be harvested without damaging fish habitat yet allowing the benefits of increased primary and secondary production and extended growing season.

Most studies of effects of logging in the Pacific Northwest and British Columbia have limited application in Alaska where logging practices, climate, geomorphology, and vegetation are different. For example, high-lead yarding is the most common logging practice in Alaska, whereas a variety of yarding methods, including skyline yarding, are used in Oregon and Washington. The cool Alaskan climate may eliminate the need to protect most streams from excessive temperature increases after logging, increases which in Oregon can kill salmonids (Moring and Lantz 1975). Buffer strips protect fish habitat in western Oregon and northern California (Hall and Lantz 1969; Newbold et al. 1980), but in Alaska, the effectiveness of buffer strips is uncertain.

Objectives of this paper are to assess short-term (<15 yr) effects of clear-cut logging, with and without buffer strips, on density of juvenile salmonids that rear in streams in southeastern Alaska and to identify habitat features that mediate these effects. To assess the effects of logging, we compare fish densities and habitat in old-growth and logged areas. We then relate fish density to habitat to identify habitat features that account for effects of logging and the importance of buffer strips.

Methods

Experimental Design

This study was based on an extensive posttreatment comparison (Hall et al. 1978) of fish densities and habitat in stream reaches with three types of treatments (Fig. 1): undisturbed forest (old-growth), clearcuts with streamside buffer strips (buffered), and clearcuts without buffer strips (clear-cut). Study streams were selected to fit a randomized, complete block design. A block consisted of a set of three streams, one of each treatment, preferably in the same watershed. The stream in each treatment area was divided into three sections of equal length, and one 30-m study reach was randomly selected from each section.

Six blocks were selected to cover regional variation in southeastern Alaska: two northern blocks on Chichagof Island, two central blocks on Kuiu and Mitkof Islands, and two southern blocks on Prince of Wales Island (Fig. 1). All three treatments, however, were usually not present in the same watershed, and five of the six blocks were composed of adjacent watersheds. All six blocks were sampled once in summer (June–August 1982) for fish and habitat, and all except Mitkof Island were sampled again for fish in winter (February–March 1983).

Characteristics of the Streams

The study streams were second to fourth order (Strahler

1957), had a low-flow discharge of 0.01−0.3 m^3/s, and had a channel gradient of 0.1−3.0%. Maximum water temperature recorded periodically in summer ranged from about 13 to 17°C in the northern and southern streams, respectively. Winter temperature ranged from 0 to 2°C. Streambed substrate consisted mostly of gravel and small cobble. Most streams had juvenile coho salmon (*Oncorhynchus kisutch*) and Dolly Varden, which were usually the most abundant fishes. About half the streams also had juvenile steelhead trout or cutthroat trout. Although common, sculpins (*Cottus aleuticus* and *C. asper*) were omitted from our analyses.

Old-growth reaches were, in all cases, located in mature forests of western hemlock and Sitka spruce, and none had any logging upstream. Buffered reaches were on the edge of 3- to 10-yr-old clearcuts: one bank had a strip of uncut streamside trees; the other had undisturbed forest (Fig. 1). In some, a logging road abutted the buffer strip, or a bridge crossed the stream. Buffer strips were 15−130 m wide and 300−600 m long, and some were selectively logged. About half the buffered reaches had one or two trees that had apparently fallen into the stream after logging. Several buffered reaches had more blowdown, and along one reach in the Shaheen Creek block, the buffer strip had mostly blown down. The clear-cut reaches flowed through 1- to 12-yr-old clearcuts where trees had been cut to both stream banks (Fig. 1). The one exception, clear-cut reaches in the Big Creek block, Mitkof Island, had undisturbed forest along one bank.

Habitat Measurements

We quantified key habitat features that may affect densities of juvenile salmonids: stream size and gradient, sediment, forest canopy, undercut banks, channel stability, pool volume, and volume of large woody debris. We also assessed primary and secondary production by measuring standing crops of periphyton and benthos. Johnson and Heifetz (1985) give details of our methods.

Habitat features at each reach were measured once in summer. We estimated stream discharge by measuring cross-sectional area and water velocity across a uniform glide. Channel gradient was measured with a hand-held level and stadia rod. At 3-m intervals along the reach, we measured stream width and estimated amount of fine sediment (<2 mm) by measuring the distance it covered across these transects. We used a hand-held convex mirror (Lemmon 1956) to estimate canopy density over the middle of the stream. Area of undercut banks was computed by multiplying length of undercut by average width of overhang. We assessed channel stability with Pfankuch's (1975) index. Pool volume was calculated by the formula for a rectangular solid from measurements of length, width, and depth of each pool. Total pool volume was standardized by dividing it by stream area.

All pieces of large woody debris (>10 cm diameter and >1 m long) within the annual high-water level of the channel were measured. The portion of each piece submerged or floating in the stream was visually estimated. Volume of boles was calculated by the Smalian formula (Chapman and Meyer 1949), and volume of root wads was calculated by the formula for a frustum. Volume of instream debris was standardized by dividing it by stream area.

Periphyton biomass per unit area of stream bed substrate was estimated from four samples of single cobbles (10−30 cm diameter) randomly selected from riffles. Attached periphyton was scrubbed from the cobbles into 50-mL jars and preserved in 5% formaldehyde. In the laboratory, the supernatant was decanted after the periphyton settled, and samples were dried (48 h, 60°C) and ashed (4 h, 550°C) to determine ash-free dry matter. To determine surface area of the cobbles, we measured three orthogonal axes of each cobble and calculated surface area by the formula for a rectangular solid. Because periphyton was concentrated on the exposed, upper half of the cobbles, only one half of total surface was used as the reference area for biomass.

In each reach, density of benthic invertebrates was estimated from four samples randomly selected from riffles. A modified Hess sampler (area 170 cm^2) was forced into the stream bed, and sediment to a depth of 10 cm was excavated into a bucket. The sample was then agitated with water, and invertebrates were decanted onto a 280-μm-mesh net and combined with those from a 280-μm-mesh net attached to the sampler. Invertebrates were preserved in 10% formaldehyde and later counted under a microscope.

Salmonid Populations

Populations of all salmonids in each reach were estimated by single-census mark and recapture (Robson and Regier 1971). We enclosed fish within each reach by blocking both ends with seines. Fish were collected with seines, electroshockers, and traps baited with salmon roe (Bloom 1976). Captured fish were anesthetized with dilute Tricaine solution, identified, measured, weighed, and marked by removal of a tip of the caudal fin. After the fish recovered from anesthesia, they were released in the same area as captured. Following the practice of Peterson and Cederholm (1984), we waited at least 1 h before attempting recapture.

Population estimates were calculated separately for fry (age 0) and parr (age >0), which were separated primarily on the basis of length. Coho fry, for example, ranged from 31 to 71 mm in summer, compared with 45 to 117 mm for coho parr. To help separate coho salmon fry from parr from streams where their sizes overlapped, we took scales from about 30 coho salmon whose lengths spanned the region of length overlap, determined age from the scales, and divided 5-mm size classes of salmon proportionately. Fish density was standardized by dividing population estimates by the area of stream in summer.

Following guidelines in Robson and Regier (1971), we tried to mark (M), recapture (R), and examine for marks (C) enough fish so that population estimates ($\hat{N}$) would not depart from the true population size by more than 25%, 95% of the time. This level of precision, however, was difficult to achieve in practice because many populations were small, and some species and age-classes were difficult to capture. The average 95% confidence interval for 320 estimates of coho salmon fry and parr, Dolly Varden parr, and trout parr populations in summer and winter (estimates with $M = 0$ or $C = 0$ omitted) was $\hat{N} \pm 58\%$.

We only considered data for coho salmon fry and parr, Dolly Varden parr, and trout parr (steelhead and cutthroat trout combined). Dolly Varden fry and trout fry emerged late and were difficult to capture; hence, few estimates of their populations were reliable. Total density of salmonid parr was strongly correlated with density of Dolly Varden parr in both summer ($r = 0.95$, $n = 54$) and winter ($r = 0.95$, $n = 45$) and therefore was excluded from the analysis.

Data Analysis

The data analysis was divided into two parts with different

TABLE 1. Habitat variables included in the regression analysis.

Habitat variable	Definition
Stream width (m)	Mean stream width
Channel gradient (%)	Percent change in elevation of reach, from bottom to top, relative to reach length
Fine sediment (%)	Percent of lateral-transect length that covered sediment <2 mm diameter
Channel instability (nondimensional)	Pfankuch (1975) index, high values indicate low stability
Undercut banks (m^2)	Total area of undercut bank
Canopy density (%)	Percent canopy closure, based on method of Lemmon (1956)
Pool volume ($m^3/100\ m^2$)	Volume of pools per 100 m^2 of stream area
Instream debris ($m^3/100\ m^2$)	Volume of instream large woody debris per 100 m^2 of stream area
Periphyton biomass (mg AFDM/m^2)	Mean ash-free dry weight of periphyton per square metre of stream bed in riffles
Benthos density (thousands/m^2)	Mean no. of benthic marcoinvertebrates per square metre of stream bed in riffles

TABLE 2. Number of reaches with reliable population estimates by block and treatment in summer (S) and winter (W) that were included in the regression analysis. Population estimates in these reaches had at least one fish marked and released ($M > 0$) and at least 15% marked fish in the recapture sample ($R/C > 0.15$).

	Coho salmon				Dolly Varden parr		Trout parr	
	Fry		Parr					
	S	W	S	W	S	W	S	W
Block								
Chichagof Island								
Kennel Creek	5	7	6	6	8	6	3	6
Corner Creek	6	5	9	8	6	4	8	8
Kuiu Island								
Straight Creek	0	4	9	4	5	4	0	0
Prince of Wales Island								
Gutchi Creek	6	7	7	7	5	6	7	6
Shaheen Creek	8	9	9	9	7	8	7	6
Treatment								
Old-growth	7	11	12	12	9	10	12	11
Buffered	9	12	14	9	9	7	5	6
Clear-cut	9	9	14	13	13	11	8	9
Total	25	32	40	34	31	28	25	26

objectives. First, to detect effects of logging on fish density and habitat, we used analysis of variance (ANOVA), with treatment, block, and region (southern, central, and northern) as factors. Second, to identify possible causes of treatment effects on fish, we used multiple regression analysis to examine associations between fish density and habitat.

The ANOVA was based on a mixed model, with random block effects, fixed treatment and region effects, and with repeated observations on streams (Winer 1971) in their lower, middle, and upper sections. Within-stream means and standard deviations for each variable were examined to determine if the variance was associated with the mean. If so, data were transformed to square roots or logarithms based on the slope of the regression of the logarithm of the standard deviation on the logarithm of the mean (Dixon et al. 1983). We transformed data for fish densities, stream gradient, sediment, undercut bank area, pool volume, debris volume, and periphyton biomass to square roots and transformed stream width and benthos density to logarithms. Planned a priori *t*-tests were used to compare the old-growth treatment with the buffered and clear-cut treatments. We included data from all reaches, regardless of precision of population estimates, to avoid any bias in data selection. For winter fish densities, however, only one central block, Kuiu Island, was sampled; therefore, we excluded data from this block to balance the design for the ANOVA.

In the multiple regression, we regressed fish density on 10 habitat variables (Table 1) by forward step-wise procedure (Kim and Kohout 1975) until all significant ($P < 0.05$) variables were added. Regression models that included data from all reaches often explained little of observed variation in fish density and had low coefficients of determination ($R^2 < 0.3$). To more reasonably evaluate the regression models, we included only those population estimates with good reliability: $M > 0$ and $R/C > 0.15$ (Table 2). For example, the Big Creek block, Mitkof Island, was deleted because the electroshocker malfunctioned and population estimates were unreliable. In summer, some streams were sampled before most coho salmon fry had emerged, which added excessive variance and made the regression analysis inconclusive. Therefore, in the regression analysis of summer fry density, we included only data from reaches where mean length of fry was >40 mm. The number of reaches used in the analysis ranged from 25 to 40 for summer coho salmon fry and parr, respectively (Table 2). Reaches were well distributed among the five blocks and the three logging treatments, except there were few buffered reaches with trout parr.

Results and Discussion

Fish densities and habitat often differed significantly between the old-growth, buffered, and clear-cut reaches, but treatment effects were frequently inconsistent among blocks. Such inconsistency probably resulted from variable stream characteristics and logging practices among the six study locations. The treatment × region interaction was nearly significant at $P < 0.15$ for periphyton biomass, summer density of coho salmon fry, and winter density of coho salmon parr. For these, treatments tended to have different effects in the southern, central, and northern regions. Treatment effects on all other variables were similar in the three regions.

Effects on Habitat

Although treatments were similar in stream width, channel gradient, and percentage of fine sediment, clear-cut reaches had less undercut bank, canopy density, pool volume, and debris and more periphyton than old-growth reaches (Table 3). Clear-cut reaches were also less stable than old-growth reaches, having significantly greater point-bar formation, sedi-

TABLE 3. Comparison of habitat in old-growth, buffered, and clear-cut reaches. Data are means of habitat variables measured in 18 reaches of 6 streams for each treatment (95% confidence intervals are in parentheses). Significance is based on a mixed-model analysis of variance, with fixed treatment and region effects and random block effects, as explained in Methods. Asterisks show results of a priori *t*-tests: treatment different from old growth, $*P < 0.10$; $**P < 0.05$; $***P < 0.01$.

Habitat variable	Old-growth	Buffered	Clear-cut	Treatment × region interaction *P*	Treatment effect *P*
Stream width (m)	5.8 (3.9–8.5)	7.0 (4.8–10.2)	4.9 (3.3–7.2)	0.39	0.35
Channel gradient (%)	1.3 (0.9–1.7)	1.2 (0.9–1.7)	1.2 (0.8–1.6)	0.28	0.88
Fine sediment (%)	7.9 (3.1–14.8)	11.2 (5.2–19.2)	7.8 (3.0–14.7)	0.79	0.61
Channel instability	68.9 (61.5–76.3)	75.5 (68.1–76.3)	91.9*** (84.5–99.2)	0.33	0.004
Undercut banks (m^2)	6.0 (4.7–9.5)	4.2 (2.9–7.7)	2.2** (0.6–5.4)	0.81	0.08
Canopy density (%)	73.8 (61.2–86.4)	64.6 (51.9–77.2)	26.7*** (14.1–39.3)	0.40	0.002
Pool volume (m^3/100 m^2)	21.4 (17.3–26.0)	16.6 (13.0–20.6)	9.4*** (6.7–12.5)	0.34	0.003
Instream debris (m^3/100 m^2)	2.2 (1.1–3.6)	4.0* (2.5–5.9)	1.0* (0.4–2.0)	0.28	0.02
Periphyton biomass (mg AFDM/m^2)	2.0 (1.2–3.2)	2.9 (1.8–4.7)	4.6** (2.9–7.4)	0.14	0.06
Benthos density (thousands/m^2)	5.0 (2.6–9.6)	7.8 (4.1–15.1)	7.9 (4.1–15.5)	0.32	0.43

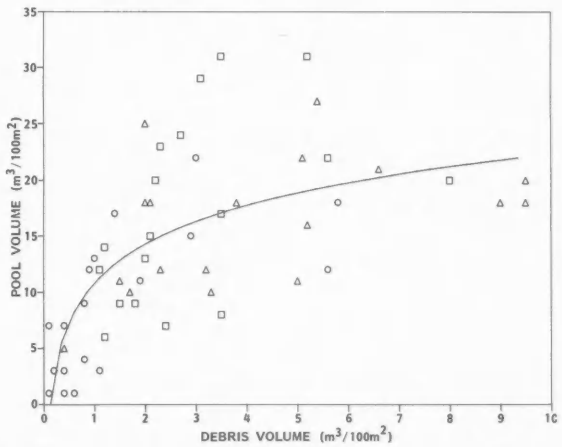

FIG. 2. Relationship between pool volume and debris volume in the old-growth (□), buffered (△), and clear-cut reaches (○). The regression equation was pool volume = 10.8 + 5.0 ln (debris volume); $R^2 = 0.47$, $n = 54$ reaches, $P < 0.001$.

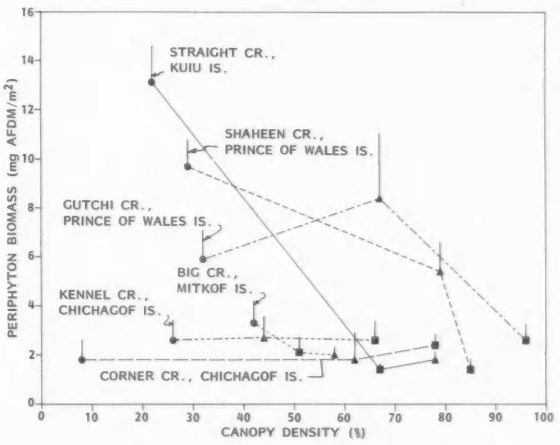

FIG. 3. Relationships between periphyton biomass and canopy density in the old-growth (■), buffered (▲), and clear-cut (●) reaches in each of the six blocks. Points are means; bars are upper halves of 95% confidence intervals for periphyton biomass. As in Fig. 1, stream names refer to the major streams in each block.

ment packing, and scour and deposition. Buffered reaches, on the other hand, had more debris than old-growth reaches but did not differ consistently from old-growth reaches in any other habitat variable.

Compared with that in old growth, debris volume was greater in buffered reaches but less in clear-cut reaches (Table 3; Fig. 2). Although buffered reaches averaged 82% more debris than old-growth reaches, this difference was mainly due to blowdown in only a few reaches. Clear-cut reaches averaged 54% less debris than old-growth reaches, but debris volume varied. Some clear-cut reaches had apparently been overcleaned of debris after logging; natural as well as logging debris had been removed, and debris was virtually absent. In a few reaches, however, much natural debris remained, and debris was as abundant as in old growth. Because of the variability in treatment effects, the differences in debris volume between logged and old-growth reaches were only mildly significant ($P < 0.10$, *t*-test).

Most pools were formed by debris, and pool volume and debris volume were directly related (Fig. 2). The few buffered reaches with heavy blowdown had the most debris, but pool volume, although large, was similar to that in some old-growth reaches with less debris. The new debris from blowdown had probably not yet produced an increase in pool volume. Clear-cut reaches had less debris than old-growth reaches, and as a result, averaged 56% less pool volume (Table 3). Where natural debris was left, however, pool volume was maintained.

Canopy density depended on logging treatment (Fig. 3). Clear-cutting to both stream banks reduced canopy to an average density of 8–32%, and clear-cutting to one bank only (Big Creek block, Mitkof Island) reduced canopy density to 42%. Buffers had variable effects on canopy, depending on buffer width. For example, in the central blocks on Kuiu and Mitkof islands, buffer strips were >100 m wide, and canopy density was greater over these buffered reaches than over comparable reaches in old growth. All other buffers were narrower (mean 45 m), selectively logged, or partially blown down, and they provided less canopy than did comparable old growth.

Periphyton biomass averaged 130% greater in clear-cut

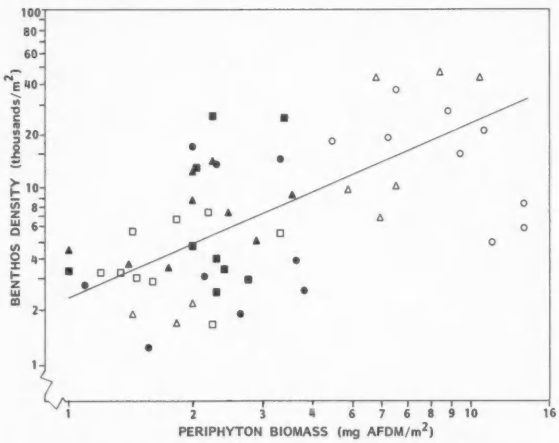

FIG. 4. Relationship between benthos density and periphyton biomass in the old-growth (□, ■), buffered (△, ▲), and clear-cut reaches (○, ●). Open symbols indicate data from Kuiu and Prince of Wales islands. Points are means of four samples of both periphyton biomass and benthos density from each reach. Both axes are in logarithmic scale. The regression equation was log (benthos) = 0.76 log (periphyton) + 0.46; $R^2 = 0.34$, $n = 54$ reaches, $P < 0.001$.

reaches than in old-growth reaches, but the treatment effect varied among the three regions (Table 3; Fig. 3). In the four southern and central blocks on Kuiu, Mitkof, and Prince of Wales islands, canopy removal appeared to increase abundance of periphyton, probably by increasing the amount of light reaching the stream (Murphy and Hall 1981). In these blocks, differences in periphyton roughly corresponded to differences in canopy. In the two northern blocks on Chichagof Island, however, periphyton biomass was unrelated to canopy; the clear-cut reaches had sparse periphyton even though they were exposed to direct sunlight.

The difference in periphyton biomass between old-growth and buffered reaches was not significant because of variation in the buffered treatment's effect on canopy density and differences among regions (Table 3; Fig. 3). The wide buffers in the central blocks on Kuiu and Mitkof islands provided as much canopy as old growth, and these buffered reaches had sparse periphyton. The buffered reaches in the southern blocks on Prince of Wales Island were more open to sunlight and had abundant periphyton. As with clear-cut reaches on Chicagof Island, the buffered reaches there had sparse periphyton even though their canopies averaged 66 and 80% of that in comparable old growth.

Periphyton biomass and benthos density were directly related (Fig. 4). The clear-cut and buffered reaches with open canopy and abundant periphyton also had abundant benthos, whereas the reaches with sparse periphyton, whether logged or old growth, generally had sparse benthos. The relationship was clearest among the reaches on Kuiu and Prince of Wales islands. Increased biomass of periphyton apparently led to high benthos density via energy transfer through the food web (Hawkins et al. 1982).

As with periphyton biomass, benthos density was usually greater (depending on canopy) in buffered and clear-cut reaches than in old-growth reaches in the southern and central blocks, but density was similar among treatments in the northern blocks. The treatment effect and treatment × region interaction, however, were not significant when all three treatments were compared in the ANOVA (Table 3) because of variation in the buffered treatment, i.e. variation in buffer width masked any treatment effect. A separate ANOVA of only old-growth and clear-cut treatments showed a significant treatment effect ($P = 0.06$) and treatment × region interaction ($P = 0.03$). Thus, in the southern and central regions, clear-cut reaches averaged significantly more benthos than did old-growth reaches (10 046/m² in clearcuts vs. 4111/m² in old growth), but in the north, there was no effect (5370/m² in clearcuts vs. 8511/m² in old growth).

Coho Salmon Fry

Summer density of coho salmon fry averaged more than two times greater in both buffered and clear-cut reaches than in old-growth reaches, but treatment effects differed among regions (Table 4; Fig. 5). As with periphyton biomass, fry density was greater in clear-cut than in old-growth reaches in the four southern and central blocks on Kuiu, Mitkof, and Prince of Wales islands, but densities were similar in clear-cut and old-growth reaches in the two northern blocks on Chichagof Island. Buffered reaches had more fry than old-growth reaches in the two southern blocks and in one northern block, Corner Creek, where they had a high density of mostly small fry that had recently emerged. Reaches with wide buffers in the two central blocks were similar to old growth.

Logging effects on summer density of coho salmon fry were related to effects on periphyton and benthos. Summer fry density was directly related to periphyton biomass and benthos density, and best modeled by periphyton biomass (Table 5; Fig. 6). The more periphyton a reach had, the more fry it had, and the clear-cut and buffered reaches with open canopy and abundant periphyton had the most fry. The correlation between fry density and periphyton biomass was much higher for the 25 reaches with fry >40 mm mean length than for all 54 reaches ($r = 0.90$ vs. $r = 0.37$). Apparently, the relationship between fry density and periphyton biomass was clearest where fry densities had the most time to adjust to the reaches' carrying capacities.

In winter, density of coho salmon fry was still greater in buffered than in old-growth reaches (Table 4). Clear-cut reaches, however, no longer had significantly more fry than old-growth reaches, and treatment effects no longer differed among regions. Differences between clear-cut and old-growth reaches changed in several blocks between summer and winter (Fig. 5). Fry density in clear-cut reaches was still greater than in old-growth reaches in only two blocks, Straight and Shaheen creeks, and was less than in old-growth reaches in the northern Corner Creek block.

Both summer food abundance and debris appeared to influence winter density of coho salmon fry. In winter, density of fry was still directly related to summer periphyton biomass and benthos density, although the correlations were not as strong as in summer (Table 5). Winter fry density was best modeled by a combination of summer periphyton biomass and volume of instream debris (Fig. 6). The more periphyton a reach had in summer and the more debris, the more fry it had in winter.

Apparently, summer food abundance limited summer fry densities, and quality of winter habitat (i.e. debris) determined winter survival. For example, fry density declined 98% (from 1.4 to 0.03/m²) from summer to winter in two clear-cut reaches in the Gutchi Creek block, Prince of Wales Island, that had abundant periphyton and benthos in summer but contained

TABLE 4. Comparison of fish density (no./100 m^2) in old-growth, buffered, and clear-cut reaches in summer and winter. Data are means of 18 reaches of 6 streams for each treatment in summer and 12 reaches of 4 streams for each treatment in winter (95% confidence intervals are in parentheses). Significance is based on a mixed-model analysis of variance, with fixed treatment and region effects and random block effects, as explained in Methods. Asterisks show results of a priori *t*-tests: treatment different from old growth, $*P < 0.05$; $**P < 0.01$.

Fish group	Old-growth	Buffered	Clear-cut	Treatment × region interaction *P*	Treatment effect *P*
		Summer			
Coho salmon fry	65.9 (27.9–120.1)	167.6* (102.0–249.3)	164.0* (99.2–244.9)	0.07	0.04
Coho salmon parr	8.6 (4.3–14.6)	10.0 (5.2–16.2)	5.6 (2.3–10.6)	0.36	0.35
Dolly Varden parr	14.5 (2.0–38.4)	39.4 (15.2–75.1)	16.4 (2.8–41.4)	0.24	0.22
Trout parr	6.0 (1.1–14.8)	1.9 (0.0–7.7)	4.4 (0.5–12.3)	0.41	0.45
		Winter			
Coho salmon fry	4.7 (1.5–9.7)	15.6* (9.1–23.8)	8.5 (4.0–14.9)	0.23	0.05
Coho salmon parr	3.8 (2.1–6.0)	4.9 (3.0–7.4)	0.2** (0.0–1.0)	0.08	0.005
Dolly Varden parr	13.4 (0.0–51.3)	17.2 (0.4–58.7)	9.0 (0.0–42.2)	0.90	0.82
Trout parr	10.4 (4.2–19.3)	3.0 (0.3–8.5)	1.8* (0.0–6.4)	0.20	0.07

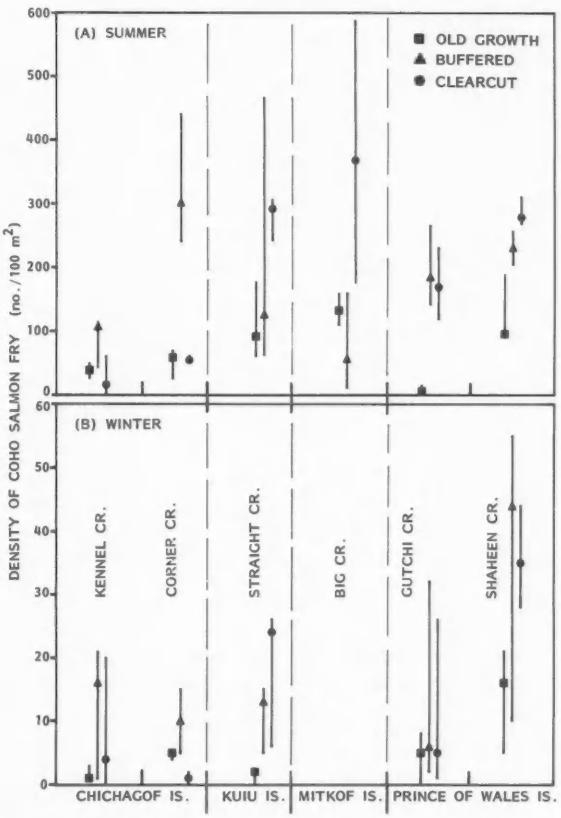

FIG. 5. Density of coho salmon fry in old-growth, buffered, and clear-cut reaches of the three streams in each of (A) six blocks in summer and (B) five blocks in winter. Points are medians; bars are ranges for the three reaches in each stream. Within a block, bars that do not overlap are significantly different ($P = 0.05$, median test). Names of the blocks refer to locations as in Fig. 1.

virtually no debris. All old-growth reaches and logged reaches in the northern blocks on Chichagof Island had sparse summer periphyton and a low winter density of fry (Fig. 6). Although some of these reaches contained a large volume of debris, the debris did not harbor many fry, presumably because fry densities were limited by low abundance of food in summer. The highest winter densities of fry were in certain buffered reaches, especially the Shaheen Creek reach with dense blowdown, where summer periphyton, benthos, and debris were all abundant.

Coho Salmon Parr

Coho salmon parr followed a different pattern of abundance from that of fry (Fig. 5 and 7). For example, in the southern and central blocks, where fry were more abundant in clear-cut than in old-growth reaches in summer, parr density in clear-cut reaches was similar to that in old-growth reaches in three blocks and less than that in old-growth reaches in one block. Thus, the greater abundance of fry in these clear-cut reaches was not associated with greater abundance of parr.

Unlike fry, parr were about equally abundant in old-growth, buffered, and clear-cut reaches in summer, but significantly less abundant in clear-cut than in old-growth reaches in winter (Table 4; Fig. 7). A mildly significant treatment × region interaction showed that logging effects in winter also tended to differ among the study locations. In the southern blocks, winter parr density in clear-cut reaches averaged only 3% of that in old growth; in the northern blocks, it averaged 76% of that in old growth, primarily because of the high density of parr in one clear-cut reach in the Corner Creek block. Without this reach, parr density in the northern block clear-cut reaches averaged only 6% of that in old growth. Buffered reaches, on the other hand, averaged 50% more parr than old-growth reaches in winter, but this difference was not significant ($P > 0.35$, *t*-test) because of variability in the buffered treatment. The high average parr density in buffered reaches was mainly due to the high density in a few reaches with dense blowdown in the Shaheen and Corner creek blocks.

Logging effects on density of coho salmon parr were related to effects on pools and debris. Summer density of parr was directly related to and best modeled by pool volume; winter density was directly related to pool volume, debris volume, and area of undercut banks and was best modeled by pool volume and debris volume (Table 5; Fig. 6). Cover in the form of debris and undercut banks was, thus, more important in winter than in summer, and reaches with the most pools, debris, and undercut banks harbored the most parr in winter.

Old-growth and buffered reaches usually had more pools and debris than clear-cut reaches; hence, most of the lowest winter parr densities were in clear-cut reaches where pool volume was low because debris had apparently been removed or washed from the reaches (Fig. 6 and 7). One of the highest winter densities, however, was in the clear-cut reach in the Corner Creek block where much natural debris remained and pool volume was high. The buffered reaches in the Shaheen and Corner creek blocks, with dense blowdown, harbored the highest and third-highest densities of parr in winter. Thus, treatment effects on winter parr density varied probably because of variable blowdown in buffer strips, as well as variable salvage and cleanup of debris from clear-cut reaches. Where debris was abundant, parr were abundant; where debris was removed, parr were scarce.

Dolly Varden Parr

Although average density of Dolly Varden parr was about two times greater in buffered than in old-growth and clear-cut reaches, treatment effects were not significant because they varied inconsistently among blocks (Table 4; Fig. 8). In winter, for example, buffered reaches had more parr than old-growth reaches in three blocks, but fewer parr in two blocks. Clear-cut reaches had more parr than old-growth reaches in one block, but fewer parr in another block. Treatment effects appeared to be masked by inherent differences in abundance of Dolly Varden among streams within blocks. For example, in the central blocks, Straight and Big creeks, Dolly Varden were absent from all the old-growth reaches yet were abundant in the buffered reaches. This difference existed in spite of these reaches having wide buffer strips and an appearance similar to old growth.

In both summer and winter, density of Dolly Varden parr was directly related to debris volume (Table 5) and was best modeled by a combination of debris volume and channel stability (Fig. 6): the more debris and the greater the stability, the higher the density of parr. In the buffered reaches with blowdown, high densities corresponded to large volumes of debris. The low densities of parr in most clear-cut reaches reflected low volumes of debris and low channel stability (Table 3). Thus, as with coho salmon parr, variable treatment effects on density of Dolly Varden parr were related to variable effects on habitat, especially debris.

Trout Parr

Density of trout parr did not differ significantly between old-growth and logged treatments in summer, but was significantly lower in clear-cut than in old-growth reaches in winter (Table 4). Although buffered reaches had only about one-third the trout density in old growth, this difference was not significant because of inconsistent differences among blocks (Fig. 9). In summer, for example, buffered reaches had fewer trout parr than in old growth in two blocks, but similar densities in four blocks. Trout density also differed inconsistently between clear-cut and old-growth reaches in summer, but density was lower in clear-cut than in old-growth reaches in nearly every block in winter. Trout density in clear-cut reaches was 73% of that in old growth in summer, but only 17% of that in old growth in winter.

TABLE 5. Product-moment correlations between fish density and habitat variables in summer and winter (*significant at <5% level; significance level was set at $\alpha/10$, i.e. with significance level α divided by the number of tests made for each fish variable). Reaches included are those with reliable population estimates, as described in Methods.

Habitat variable	Coho salmon		Dolly Varden	Trout
	Fry	Parr	parr	parr
		Summer		
Number of reaches	25	40	31	25
Stream width	0.32	0.00	−0.17	−0.65*
Channel gradient	−0.42	0.24	0.30	0.04
Fine sediment	−0.37	0.15	0.16	−0.18
Channel instability	0.40	0.17	−0.40	−0.32
Undercut banks	−0.02	0.15	0.13	0.19
Canopy density	0.00	0.20	0.12	0.02
Pool volume	−0.33	0.81*	0.20	−0.24
Instream debris	−0.03	0.38	0.46*	−0.24
Periphyton biomass	0.90*	−0.27	−0.41	0.00
Benthos density	0.67*	−0.55*	−0.43	−0.05
		Winter		
Number of reaches	32	34	28	26
Stream width	0.54*	0.35	0.18	−0.07
Channel gradient	0.03	0.25	0.34	0.27
Fine sediment	−0.17	0.10	0.11	−0.44
Channel instability	0.38	0.16	−0.11	−0.24
Undercut banks	0.02	0.45*	−0.06	0.57*
Canopy density	−0.20	0.38	0.35	0.48*
Pool volume	0.18	0.79*	0.30	0.30
Instream debris	0.28	0.56*	0.77*	0.29
Periphyton biomass (summer)	0.63*	−0.29	−0.38	−0.16
Benthos density (summer)	0.55*	−0.36	−0.32	−0.23

In summer, density of trout parr was significantly correlated with only one habitat variable — stream width (Table 5). Trout were less abundant in the larger streams (Fig. 6). The 12 old-growth and 8 clear-cut reaches in our subsample of reaches with reliable population estimates varied widely in both trout density and stream width, but the 5 buffered reaches had few trout, corresponding to their large stream widths. Thus, the low density of trout parr in buffered reaches (Table 4) may have been an artifact of the relatively large size of buffered streams in our sample.

Treatment effects on winter density of trout parr were related primarily to effects on undercut banks. In winter, density was no longer correlated with stream width, but was directly related to area of undercut banks (Table 5) and was best modeled by a combination of undercut banks and sediment: reaches with extensive undercut banks and little sediment had more trout parr in winter (Fig. 6). Old-growth reaches contained the highest winter densities of trout parr, which reflected their large areas of undercut banks and low amounts of fine sediment (Table 3). Buffered reaches varied widely in both trout density

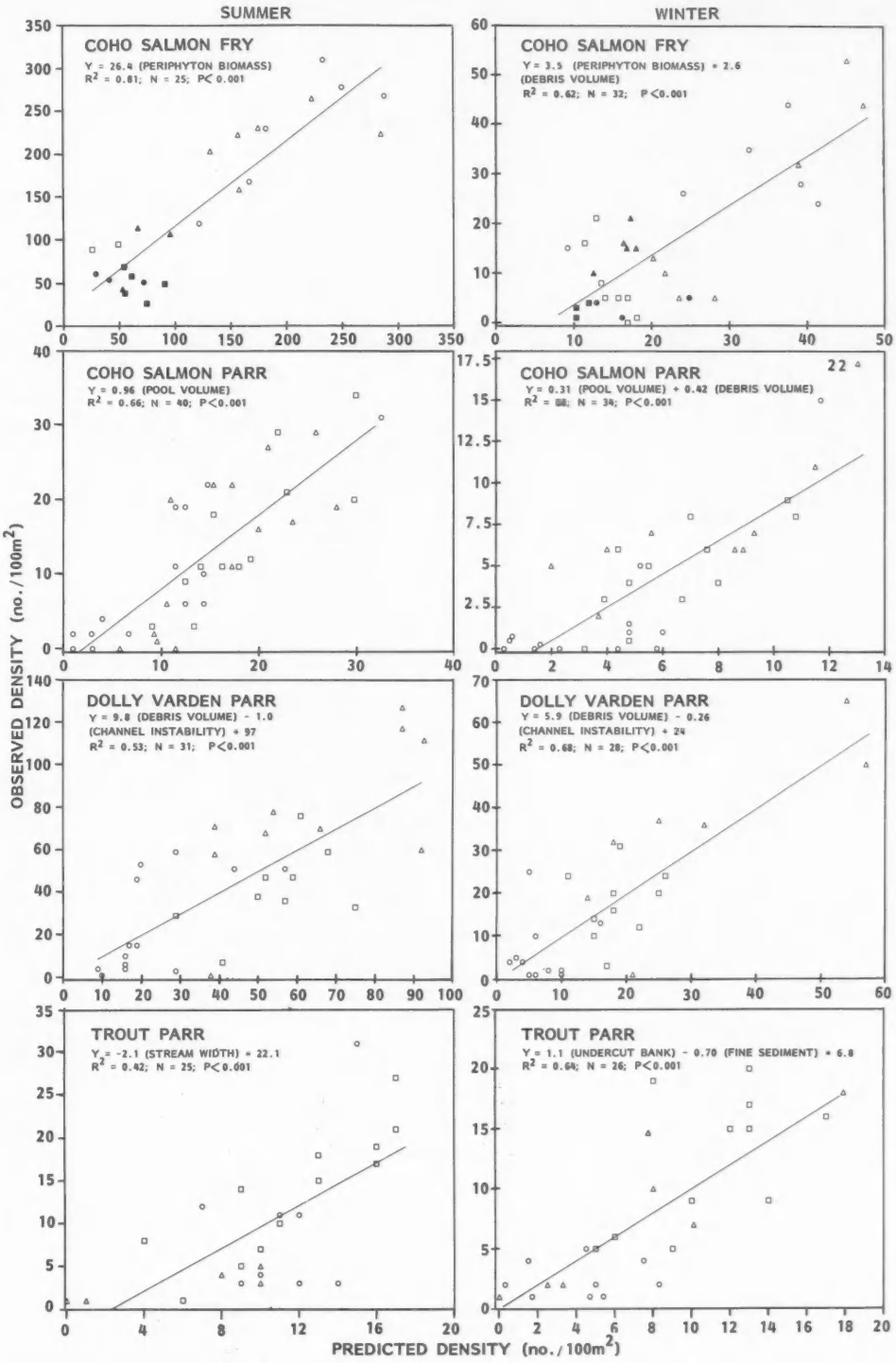

FIG. 6. Relationships between density of fishes in the old-growth (□), buffered (△), and clear-cut (○) reaches in summer and winter and that predicted by regression equations. Units of habitat variables are in Table 2. For coho salmon fry, solid symbols indicate reaches on Chicagof Island and open symbols indicate reaches on Kuiu and Prince of Wales islands.

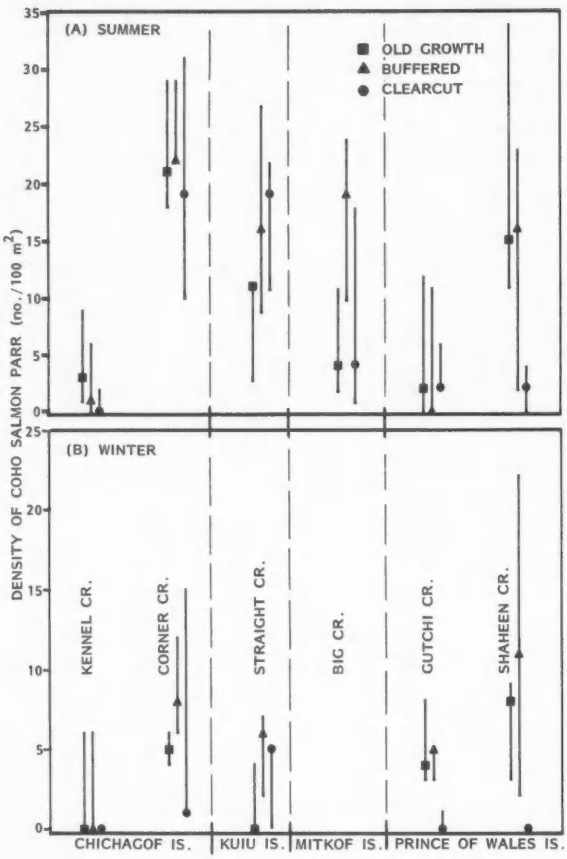

FIG. 7. Density of coho salmon parr in old-growth, buffered, and clear-cut reaches of the three streams in each of (A) six blocks in summer and (B) five blocks in winter. Points are medians; bars are ranges for the three reaches in each stream. Within a block, bars that do not overlap are significantly different ($P = 0.05$, median test). Names of the blocks refer to locations as in Fig. 1.

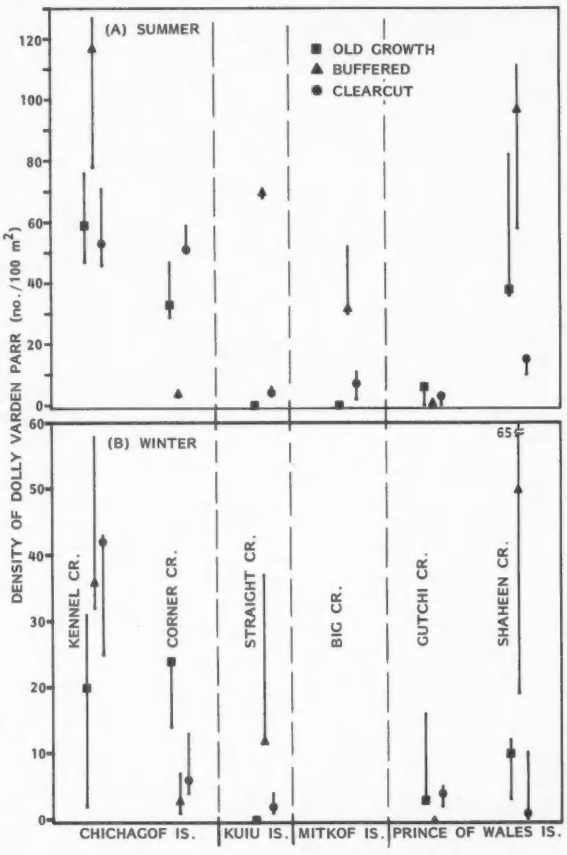

FIG. 8. Density of Dolly Varden parr in old-growth, buffered, and clear-cut reaches of the three streams in each of (A) six blocks in summer and (B) five blocks in winter. Points are medians; bars are ranges for the three reaches in each stream. Within a block, bars that do not overlap are significantly different ($P = 0.05$, median test). Names of the blocks refer to locations as in Fig. 1.

and undercut banks, whereas all clear-cut reaches had low trout density, which corresponded to their small areas of undercut banks.

General Discussion

Effects of logging on juvenile salmonids can probably be best understood as a response to simultaneous, varied changes in both physical habitat and stream productivity, changes that affect different species and life stages of salmonids differently. In this study, amounts of periphyton, benthos, pools, debris, and undercut banks all differed between old-growth and logged reaches, but logging effects often varied depending on local conditions and logging practices. Effects of logging on salmonids were associated with these varied effects on habitat.

Logging often increases stream productivity for the first 10–15 yr because canopy removal increases the amount of light reaching the stream bed. In western Oregon and northern California, for example, logging increased aquatic primary production, benthos density, and fish density (Hansmann and Phinney 1973; Lyford and Gregory 1975; Newbold et al. 1980; Murphy and Hall 1981; Hawkins et al. 1983). Similarly, shade of the old-growth forest canopy limited primary production in a stream in southeastern Alaska (Murphy 1984; Walter 1984). Low nutrients, however, can also limit primary production and preclude increased productivity after logging. Although logging increased the amount of light reaching Carnation Creek, primary and secondary production did not increase; production remained limited by low phosphorus (Shortreed and Stockner 1982; Culp and Davies 1983).

Changes in stream nutrients after logging vary. Nitrate concentration increased after clear-cutting in New Hampshire to more than 50 mg/L (Bormann et al. 1968) and in Oregon to 2 mg/L (Brown et al. 1973). After logging, nitrate concentration in Carnation Creek, British Columbia, increased to about 0.6 mg/L, but phosphate concentration remained extremely low at 0.003–0.004 mg/L (Scrivener 1982). In southeastern Alaska, clear-cutting and burning the watershed of a small stream on Chichagof Island altered neither nitrate nor phosphate, which averaged only 0.07 and 0.009 mg/L, respectively (Stednick et al. 1982).

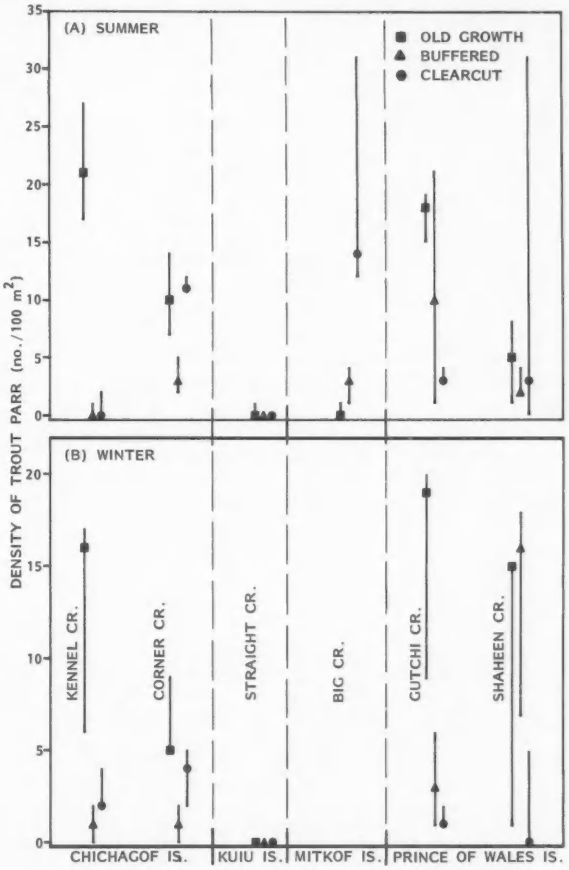

FIG. 9. Density of trout parr in old-growth, buffered, and clear-cut reaches of the three streams in each of (A) six blocks in summer and (B) five blocks in winter. Points are medians; bars are ranges for the three reaches in each stream. Within a block, bars that do not overlap are significantly different ($P = 0.05$, median test). Names of the blocks refer to locations as in Fig. 1.

Nutrient limitation may also explain why, in our study, logged reaches on Chicagof Island did not have more periphyton and benthos than reaches in nearby old-growth forest. Differences in response to canopy removal reflected the type of bedrock in the watersheds. The three blocks on Kuiu and Prince of Wales islands, with much more periphyton, benthos, and coho salmon fry in clear-cut than in old-growth reaches, had mostly sedimentary bedrock rich in limestone, whereas the two blocks on Chichagof Island, with little periphyton in logged reaches, had mostly igneous bedrock (Dutro and Payne 1954). Similarly, primary production was higher in clear-cut reaches of streams on Prince of Wales Island than in old-growth reaches of a stream on Etolin Island, southeastern Alaska (Walter 1984), both areas with limestone. However, primary production differed inconsistently between clear-cut and old-growth reaches on Chichagof Island (Weber 1981) in an area with mixed igneous and sedimentary rock.

Streams underlaid with limestone often have high concentrations of dissolved compounds, including phosphorus — a nutrient whose low concentration sometimes limits plant growth — whereas streams in areas underlaid with igneous bedrock are poor in dissolved compounds (Golterman 1975). Thus, primary production may have been limited by light in watersheds with limestone and by nutrients in watersheds with igneous rock. Periphyton response to canopy removal, however, also followed a climatic gradient from greatest response in the southern and central streams to least response in the northern streams. Since we did not measure nutrients in the streams, we can only speculate on what caused the inconsistent effects of logging on periphyton biomass.

Differences in sampling time did not account for differences in periphyton between blocks. All streams within the three pairs of blocks — Straight and Big creeks, Gutchi and Corner creeks, and Shaheen and Kennel creeks — were sampled within 1 wk of each other, yet the clear-cut reaches within these pairs had very different amounts of periphyton (Fig. 3). Periphyton sampled at different times, therefore, differed less than periphyton sampled at different locations.

Summer density of coho salmon fry in many streams appears to be limited by low sunlight and availability of food (Chapman and Knudsen 1980; Hawkins et al. 1983; Bisson and Sedell 1984). Amounts of periphyton and benthos are only indirect measures of food availability for fish, yet the direct relationship between them and fry density in our study indicates that increased abundance of periphyton and benthos in some buffered and clear-cut reaches resulted in fry densities that were twice as high as in old-growth reaches. The high fry density in these logged reaches apparently resulted from a lower rate of emigration, as in experimental streams studied by Mason and Chapman (1965), because of reduced fry aggression and territory size (Dill et al. 1981).

Differences between treatments in fry density were probably not caused by differences in egg deposition by spawners. Treatment reaches within blocks were similar in distance from the stream mouth and were equally accessible to adult salmon. Furthermore, coho salmon fry may move long distances as they disperse from the spawning site (e.g. Crone and Bond 1976). Thus, recruitment of fry was potentially the same for all treatment reaches within the same block.

The benefit of increased productivity in clear-cut reaches could be nullified if fish die during winter because favorable habitat is lacking. For example, Mason (1976) increased the summer density of coho salmon fry in Sandy Creek, British Columbia, by feeding them, but the increase was nullified by mortality the next winter. In our study, the higher summer density of coho salmon fry in some buffered and clear-cut reaches, compared with old-growth reaches, was still apparent the next winter. In the clear-cut reaches from which debris was removed, however, fry density was reduced to less than that in comparable old-growth reaches. The winter of 1982, furthermore, was mild in southeastern Alaska. In more severe winters, mortality should be greater and debris more important.

Coho salmon in southeastern Alaska usually spend two winters in freshwater before going to sea (Crone and Bond 1976; Gray et al. 1981). If our data for the two age-classes, fry and parr, are viewed as a single cohort over 2 yr (i.e. assuming stable age structure), then the greater density of fry in the clear-cut reaches would be reversed during the fish's second winter in the stream. We did not, however, follow a year-class through both years and cannot be sure that a single cohort would follow this trend. Densities of coho salmon fry can vary greatly from year to year in a stream, but by the second summer, densities of parr may be consistently reduced to a narrow

range (Crone and Bond 1976). Thus, higher mortality or greater emigration probably caused the low winter density of coho salmon parr in most clear-cut reaches.

In clearcuts, this lower parr density could also be explained if fast-growing fry became smolts after only 1 yr. Average fry length did differ significantly among the old-growth, buffered, and clear-cut reaches in late winter (51, 54, and 56 mm, respectively; J. F. Thedinga, Auke Bay Laboratory, P.O. Box 210155, Auke Bay, AK 99821, unpubl. data). Thus, a tendency for fry to become smolts after only 1 yr could have contributed to the reduction in densities of older age-classes in the clear-cut reaches. The most likely explanation for lower parr densities in clear-cut reaches, however, is the reduction in preferred winter habitat. Most clear-cut reaches had few pools, scarce debris, and few parr, but some with good pool habitat and debris had many parr. Thus, loss of pools and debris, and not younger smolt age, probably accounts for the low winter density of parr in most clear-cut reaches.

Cover in the form of debris and undercut banks was more important for parr in winter than in summer, which probably explains why logging effects on parr density were significant in winter but not in summer. At summer temperatures, food abundance may override cover in determining abundance and distribution of parr within a stream (Wilzbach 1985). Studies on the impacts of logging on salmonids, therefore, may be misleading if data are taken only during summer.

Clear-cutting without buffer strips appears to reduce winter carrying capacity for salmonid parr by removing debris, collapsing undercut banks, and destabilizing or embedding channel substrate. This conclusion is supported by other studies. After logging, winter carrying capacity for coho salmon in Carnation Creek decreased as unstable debris washed downstream (Tschaplinski and Hartman 1983). Experimental removal of debris from a small stream in southeastern Alaska reduced Dolly Varden density by about 95% (Elliott 1986). Removal of undercut banks that provided cover for <2% of a stream in Montana reduced trout density by one third (Boussu 1954). Destabilization of a stream channel in western Washington by experimental removal of debris reduced winter trout density by one third (Lestelle and Cederholm 1984). Blowdown in buffer strips can uplift root masses and eliminate undercut banks, but in these cases, the added cover of debris may substitute for the lost cover of undercut banks (Heifetz et al. 1986).

Apparently, pools are lost from clear-cut reaches as natural debris is removed when logs are salvaged, logging slash is cleared from the stream channel, or destabilized debris washes downstream or floats onto the stream banks (Toews and Moore 1982; Bryant 1983; Bilby 1984). To prevent loss of pools and debris and to preserve channel stability, improved regulations are needed to prevent disturbance or removal of debris from salmon streams during logging. New guidelines being developed (U.S. Forest Service, 709 W. 9th St., Juneau, AK 99801, unpubl. data) should help prevent such disturbance and removal of debris.

Clear-cutting without buffer strips could maintain pools and debris in the short term if natural debris is protected, as it was in a few of the reaches in our study. In the long term, however, new debris is needed to replace the debris that decays or washes out of the stream during floods (Swanson and Lienkaemper 1978; Grette 1985). Even after 60 yr, debris from second-growth forest may not be adequate to maintain fish habitat, and debris volume and pool habitat decline (Grette 1985). To maintain production of salmonids after logging, debris must be maintained, and if properly managed, streamside areas can provide the needed debris.

The management practice of leaving streamside buffer strips appears advantageous. Buffer strips allow increased primary and secondary production, protect habitat, and provide a source for additional new debris after logging. Logging with buffer strips can, in the short term, increase recruitment of fry, yet sustain survival of fry and parr through winter. In the long term, buffer strips can provide new debris for the stream and thus maintain pools and debris through the next timber harvest.

Buffer strips, however, have not been widely used in southeastern Alaska because valuable timber is left in the woods, and buffer strips sometimes blow down. Blowdown provides valuable habitat for rearing salmonids, but too many trees in a stream can block fish passage or cover spawning areas. Severe blowdown can be avoided if buffer strips are thinned or designed to resist windthrow (Steinblums et al. 1984). Research is needed to determine the number and type of trees that should be left in buffer strips that would allow harvest of some of the most valuable trees, prevent excessive blowdown, and yet maximize benefits to fisheries.

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Food Resource Utilization by Five Species of Benthic Feeding Fish in Passamaquoddy Bay, New Brunswick

J. Stevenson Macdonald[1] and Roger H. Green

Department of Zoology, University of Western Ontario, London, Ont. N6A 5B7

Macdonald, J. S., and R. H. Green. 1986. Food resource utilization by five species of benthic feeding fish in Passamaquoddy Bay, New Brunswick. Can. J. Fish. Aquat. Sci. 43: 1534–1546.

Feeding relationships of various fish species, and their relationship to the composition of the surrounding sediments, were observed for 1 yr at two sites in the lower Bay of Fundy region. The fishes were the ocean pout (*Macrozoarces americanus*, Zoarcidae), winter flounder (*Pseudopleuronectes americanus*, Pleuronectidae), plaice (*Hippoglossoides platessoides*, Pleuronectidae), cod (*Gadus morhua*, Gadidae), and witch flounder (*Glyptocephalus cynoglossus*, Pleuronectidae). After reducing the size of the data matrix by removing redundant variables, we used discriminant function analysis to assess interspecific diet overlap and the degree to which stomach contents reflect benthic composition. Variables were ranked by their power to discriminate in pairwise comparisons among fish species and between a given fish species and benthic grab samples. Many amphipods are utilized to an equal or greater extent than their abundances in the sediments would suggest. Many annelids are underutilized. Predation by each fish species was a selective process, and interspecific diet differences are related to the morphology of the predator and the behavior and microhabitat of the prey. The fishes diets, however, are also correlated with spatial and temporal changes in the benthic composition. Specific examples are presented. As benthic food abundance increased in the surrounding sediments all predator species increased their food consumption and interspecific diet overlap declined. In some cases the degree of exploitation depended upon the size rather than the abundance of the prey item.

Les relations alimentaires de diverses espèces de poissons, ainsi que leur rapport avec la composition des sédiments ambiants, ont été observées pendant 1 an à l'entrée de la baie de Fundy. Les espèces étudiées ont été la loquette d'Amérique (*Macrozoarces americanus*, Zoarcidae), la plie rouge (*Pseudopleuronectes americanus*, Pleuronectidae), la plie canadienne (*Hippoglossoides platessoides*, Pleuronectidae), la morue franche (*Gadus morhua*, Gadidae) et la plie grise (*Glyptocephalus cynoglossus*, Pleuronectidae). Après réduction de la matrice de données par enlèvement des variables redondantes, nous avons utilisé l'analyse des fonctions discriminantes pour évaluer le chevauchement des régimes alimentaires d'une espèce à l'autre et la façon dont les contenus stomacaux reflètent la composition benthique. Les variables ont été rangées selon leur pouvoir discriminant dans des comparaisons par paires entre espèces de poissons et entre une espèce de poisson donnée et des échantillons de benthos prélevés à la benne. Plusieurs amphipodes sont utilisés à un degré égal ou supérieur à ce que laisse supposer leur abondance dans les sédiments. Plusieurs espèces d'annélides sont sous-utilisées. La prédation par chaque espèces de poisson est un processus sélectif et les différences interspécifiques de régime sont fonction de la morphologie du prédateur et du comportement et du microhabitat de la proie. Il y a cependant relation entre le régime alimentaire des poissons et les changements spatiaux et temporels de la composition du benthos. Nous donnons plusieurs exemples. À mesure qu'augmente la nourriture benthique dans les sédiments ambiants, la consommation augmente chez toutes les espèces prédatrices, et il y a diminution du chevauchement interspécifique des régimes alimentaires. Dans certains cas, le degré d'exploitation dépend de la taille plutôt que de l'abondance de la proie.

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Information about food habits and food resource partitioning by northwestern Atlantic demersal fishes has been collected for more than 25 yr (Powles 1958; Tyler 1971, 1972; DeGroot 1971; Frame 1974; Bowman 1977, 1980, 1981a, 1981b; Langton and Bowman 1980, 1981). Food habits vary from region to region. Winter flounder (*Pseudopleuronectes americanus*) feed primarily on polychaetes in Long Island Sound and the Bay of Fundy (Richards 1963a, 1963b; Tyler 1972; Langton and Bowman 1981) and on mollusks and amphipods in the New England region (Frame 1974). Ocean pout (*Macrozoarces americanus*) feed on echinoderms and crustaceans in the Gulf of Maine (Hacunda 1981) and the Cape Cod (Olsen and Merriman 1946) regions, but primarily on mollusks in Passamaquoddy Bay (Tyler 1972). Plaice (*Hippoglossoides platessoides*) feed on mollusks, fishes, and crustaceans in the western Baltic (Arntz and Finger 1981) and Passamaquoddy Bay (Tyler 1972), on polychaetes off southern New England (Bowman 1981a, 1981b), and on echinoderms on the Grand Banks (J. S. Macdonald, unpublished). DeGroot (1971) described the witch flounder (*Glyptocephalus cynoglossus*) as a polychaete, mollusk, and amphipod feeder in the North Sea, but they feed primarily on annelids off the New

[1]Present address: Department of Fisheries and Oceans, 4160 Marine Drive, West Vancouver, B.C., V7V 1N6.

England states (Langton and Bowman 1981).

Demersal fishes are opportunistic feeders and therefore food habit differences among different studies are primarily the result of differences in the available food resources at each site (Pearcy and Hancock 1978; Moore and Moore 1976a). MacArthur and Pianka (1966) suggested that predators should prefer more profitable prey and be more selective when profitable prey are common. Knowledge of the benthic composition and availability within the fishes' foraging area is necessary to quantitatively evaluate the extent to which fish "select" their prey (Ivlev 1961) and to test optimal foraging, niche overlap, and niche volume theories.

Many quantitative methods have been developed to describe the degree of difference between diets of two fish species, or between diets and the composition of the food resource (Fritz 1974; Ellison 1978; Gabriel 1978; Berg 1979; Feinsinger et al. 1981). Multivariate methods have an advantage over other methods in that they estimate the variation and covariation of prey species abundances within each predator species. Tests of hypotheses about diet differences follow naturally. An eigenanalysis, such as discriminant analysis, when used in conjunction with MANOVA will allow isolation of variables (prey species) that differ among groups. However, with the exception of discriminant analysis as used by Ellison (1978) and Desselle et al. (1978), and cluster analysis as used by Ross (1977), multivariate techniques have rarely been used to compare fish diets.

This study uses multivariate techniques to explore the effect of temporal and spatial changes of the macrobenthos on food utilization and partitioning by the demersal fish community. We also considered the spatial and temporal size variation and burying behavior of macrobenthic animals because these factors affect fish food utilization patterns.

Materials and Methods

Study Sites

All fishes and benthic samples were collected along two 1.6-km transects: one in Passamaquoddy Bay (A) and the other in the Bay of Fundy (B) (Fig. 1). The sites were 55 and 80 m deep, respectively, and were relocated each collection period using a depth finder and radar. The annual temperature range was less at site B and the salinity was generally higher (Macdonald et al. 1984). Site A had stronger currents because of the tidal flushing effects of Passamaquoddy Bay. Sediment samples taken with a PONAR grab in July 1979 indicated that site B and the northern half of site A were composed of silt and clay. The other half of site A was composed of sand. Both sieve and hydrometer techniques were used to analyze the sediments (Bowles 1970). Site A is the same site sampled by Tyler (1971) during 1965 and 1966.

Data Collection

Fishes were collected using a 3/4-35 shrimp trawl constructed of 3.8-cm stretch mesh nylon with a 15.5-m head rope and towed by a 150-hp (111-kW), 14-m stern trawler. Seven replicated tows were made along each transect during the period of July 1979 to June 1980 (Table 1). Site A was not sampled during December 1979 because of the proximity of lobster trap lines. Within 3 d of each fish collection, 10 benthic samples were taken at each site using a 23-cm^2 PONAR grab.

Ten each of winter flounder, cod, plaice, ocean pout, and witch flounder were selected from the shrimp trawl (if sufficient numbers were present). Witch flounder were not caught at site A. Total length (to the nearest centimetre) and weight estimates (to the nearest gram) and sex identification were made for each specimen. To reduce the effect of fish size on food habits the size range of fish collected within each species was kept to a minimum (Table 1). Unfortunately there was a large size variation in the cod sampled. Cod become increasingly piscivorous as they grow (Powles 1958; Arntz 1974; Lilly and Fleming 1981). This increased the variability of prey species obtained from cod stomachs.

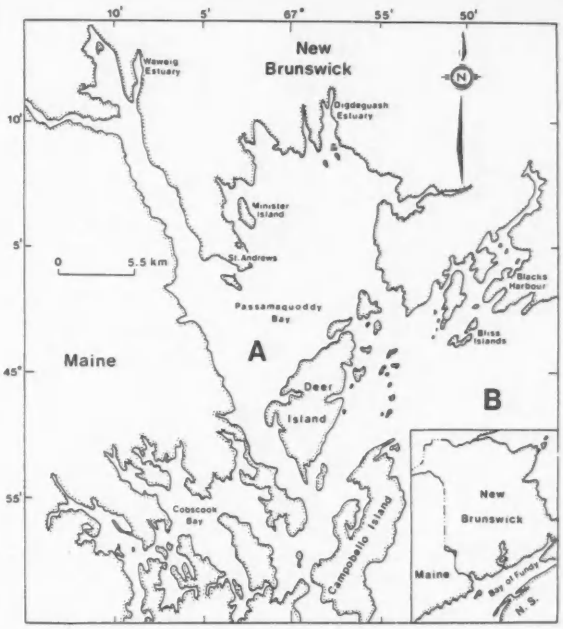


FIG. 1. Passamaquoddy Bay and the adjacent Bay of Fundy indicating sampling stations occupied during the study.

Immediately after capturing the fishes, their stomachs were removed and placed singly in numbered jars of 10% buffered formalin. In the laboratory, prey species were transferred to 70% ethanol and later keyed to the lowest possible taxonomic level. Mollusk soft parts were keyed when valves were not present. Blotted wet weight (to the nearest 0.01 g) and number of each prey species were recorded directly onto 80-column computer sheets. Otoliths were removed and stored in an equal mix of glycerin and alcohol. Fish ages were determined by the otolith aging section of the St. Andrews Fisheries and Oceans Laboratory (Table 1).

Benthic samples were washed through a 1-mm screen placed in 10% buffered formalin and then transferred to 70% ethanol in the laboratory. Laboratory analysis of grab samples was similar to that of stomach samples. In addition, three common benthic species (*Casco bigelowi*, *Leptocheirus pinguis*, and *Yoldia sapotilla*) were measured (total length) using a computer-linked measuring device (Tektronix 4954 digitizer). The technique described by Macdonald and Sudbury (1982) was followed. A two-way factorial analysis of variance (ANOVA) was used to test for differences in mean length of each species between sites and among collection times. Computing was done using the ANOVA subprogram (classical ap-

TABLE 1. Summary of numbers (n), mean lengths ($\bar{X}_L$), size deviation (S.dev), mean ages ($\bar{X}_{age}$), and sex of all fish species collected at each sampling time at sites A and B. Fish length range within each fish species was minimized and an attempt was made to collect 10 of each fish species at each site and time. This was not always possible. Each male fish was given a value of zero and each female fish a value of one. The means of all values for each species are presented to indicate the proportion of males and females collected at each site and time.

	July 18–19		Aug. 19–20		Oct. 18–19		Dec. 5	Jan. 28–31		Apr. 3–9		June 23–25	
	A	B	A	B	A	B	B	A	B	A	B	A	B
							Cod						
n	2	4	4	3	0	10	5	10	1	4	0	8	3
$\bar{X}_L$	46	74	40	75		78	93	17	49	15		27	62
S.dev.	0.63	2.8	7.2	5.8		13	19	2.8	0	1.4		7.6	15
$\bar{X}_{age}$	2	4	2	4		5	7	1	3	1		1	4
Sex	1	1		1		0.7	0.6					0.8	0
							Ocean pout						
n	10	10	10	4	10	2	6	0	1	4	1	10	6
$\bar{X}_L$	45	54	48	56	42	53	51		26	57	58	53	49
S.dev.	8.5	4.2	8.2	5.2	10	3.9	8.2		0	3.8	0	3.5	7.2
$\bar{X}_{age}$	7	8	6	8	5	7	7		3	11		8	7
Sex	0.5	0.7	0.5	0.8	0.4	0.5	0.1			0.3	1	0.2	0.8
							Winter flounder						
n	10	2	10	3	10	4	10	10	10	10	10	10	4
$\bar{X}_L$	32	37	34	40	34	38	32	25	26	28	27	28	38
S.dev.	5.9	5.1	8.0	3.2	6.6	4.4	6.0	4.3	8.8	4.4	4.5	4.1	1.1
$\bar{X}_{age}$	5	6	6	7	5	6	5	4	5	5	4	4	7
Sex	0.7	0.5	1	1	0.7	0.5	0.9	0.5	0.6	0.4	0.4	0.6	0.8
							American plaice						
n	9	10	10	10	10	10	9	3	10	8	10	10	10
$\bar{X}_L$	22	30	26	28	23	30	28	23	20	23	25	27	27
S.dev.	2.2	5.2	3.4	4.1	1.0	7.6	5.9	0.8	3	3.4	4.9	1.5	2.7
$\bar{X}_{age}$	2	7	3	4	3	4	5	3	3	3	4	3	4
Sex	1	0.5	1	0.5	0.9	0.7	0.5		1	1	0.4	0.5	0.6
							Witch flounder						
n	0	6	0	10	1	10	10	0	2	0	0	0	10
$\bar{X}_L$		49		47	60	47	39		26				54
S.dev.		11		11.1	0	11	16		1.3				2.4
$\bar{X}_{age}$		16		12	12	12	9		5				13
Sex		1		1	1	0.8	0.9		1				0.9

proach) in SPSS on a Cyber 170 at the University of Western Ontario Computing Centre.

To determine the depth distribution within the sediment for common benthic prey species, we collected 10 mud samples with a tall Eckman grab (14 × 14 × 23 cm high) at site A during June 1980. The column of mud that resulted from each grab was immediately sliced into five 2-cm sections. Each section was sieved through a 1-mm screen and stored in 10% buffered formalin. Species were identified to their lowest taxonomic level. A one-factor ANOVA followed by a Student-Newman-Keuls (SNK) multiple range test was used to examine mean depth differences among the benthic species. The depths were transformed as $\log(X + 1)$ before analysis.

Data Analysis

This is a three-dimensional multivariate study in which six sampler-types (five fish species plus the PONAR bottom grabs) were collected from two sites at seven times during a year. The dependent variables (response variables) were 217 prey or potential prey species identified from one or more of the sampler-types.

There is usually a great deal of information redundancy when the data matrix contains this many variables (Green 1979). Therefore, three methods were used to reduce the size of the matrix.

(a) Only numbers of prey present (not weight) were used as

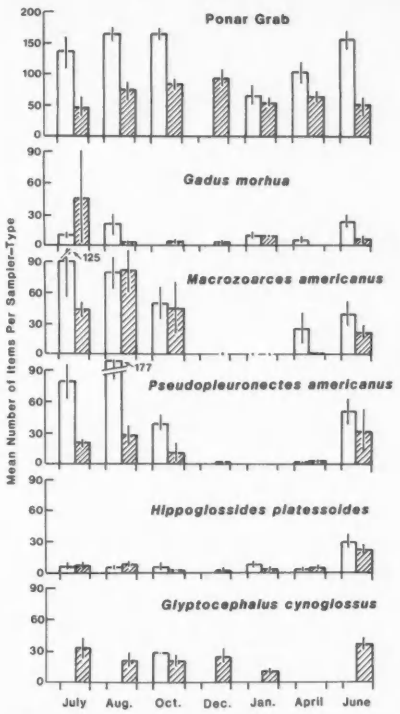


FIG. 2. Graph of mean number of individual prey sampled from each fish species and benthic individuals from ponar grabs at each collection time and site. Vertical lines indicate 1 standard error of the mean. Site B bars are hatched and site A are open. A dot without a bar indicates that fishes caught had empty stomachs.

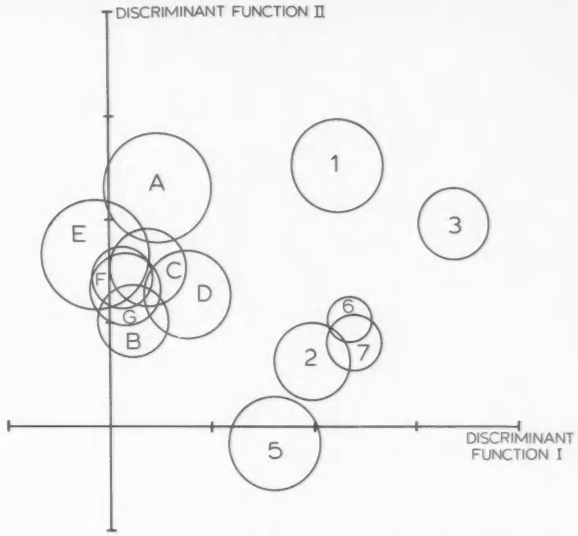


FIG. 3. Results of discriminant analysis on the benthic density data collected from each site−time combination. The discriminant scores from each of the 13 groups are contained within circles. the circles are identified by number (site A collections) or by letter (site B collections). Letters A, B, C, D, E, F, and G and numbers 1, 2, 3, 5, 6, and 7 refer respectively to collections in July, August, October, December, January, April, and June. Collections 4 (December at site A) was not made.

a variable in the analysis. Little information is gained by using more than one measurement type (Macdonald and Green 1983).

(b) A correlation technique was used to rank the variables (prey species) according to the additional information each one added to the entire data matrix (Orloci 1978, program name "Rank"). The variable given rank 1 has the highest Pearson product-moment correlation coefficient with the other variables. The variable given rank 2 has the highest correlation with the information remaining (i.e. not predicted by the rank 1 variable). In this manner, 217 variables were reduced to 126.

(c) Variables that occurred in less than 2% of the 527 cases were eliminated. This left the 74 variables that were used in the analysis. Included were most of the 22 principal prey species taken by demersal fish in Passamaquoddy Bay according to an earlier study (Tyler 1972).

A three-factor multivariate analysis of variance (MANOVA) was used to test for overall significant differences among mean prey abundance of all site−time−sampler type groups within the data matrix. Before this analysis could be done, the data from December and the data for witch flounder were removed to give a fully crossed design. A log $(X + 1)$ transformation of the abundance variables was chosen to remove dependence of the variance on the mean (Taylor's power law, Elliott 1977). Discriminant analysis (in the sense of canonical analysis; SPSS) was used to compare benthic composition among sites and times. Variables (benthic species) were ranked by F-value on their overall ability to discriminate in pairwise comparisons of benthic collections at each site and time (Jancey 1979, program called "K-RANK"). The F-value results from the ratio of between benthic collections at each site and time to within benthic collection variances (mean squares) of each variable. Variables were said to have significant discriminating power if their alpha values were less than 0.05 after Bonferroni's inequality equation was used to corrent for the increased chance of making a type one error that occurs with repeated F-test (Snedecor and Cochran 1980). Unlike the overall MANOVA test (which indicated a great deal of statistical significance), the Bonferroni corrected F-tests were used as a selection criteria, not as a formal method of testing hypotheses. While independence among tests is required for the formal use of this test, it is unlikely important in these circumstances.

Discriminant analysis was also used to estimate the significance of the discriminant functions (DF) that gave the maximum separation among the fish species (based on their diets) and the benthic composition at each site−time combination. Species that were preyed upon by each predator species to a greater or lesser degree than expected from their abundance in the sediment, at each site−time combination, were identified using the same ranking procedure used for the benthic samples (Jancey 1979). In this manner, diets of all fishes were compared with the potential food components in the sediment to estimate the degree of feeding selectivity exhibited by each predator species during each month.

Some of the difference among sampler-types, in this analysis, is due to the grab taking a larger number of organisms than the fishes (Fig. 2). However, the largest discriminant function coefficients (loadings) of the first discriminant function of each site−time analysis were generally divided between positive and negative values. This indicates that the first vector de-

TABLE 2. List of ranked log abundance variables that discriminate between benthic collections at each site using the *F*-statistic (Jancey 1979). Variables are significant at the 0.05 level after Bonfernoni's inequality equation was applied. Letters indicate site at which each variable was most abundant during each collection period. Many of the prey species that show a significant difference in abundance among collection times are listed in Fig. 5.

Discriminators (between sites, grabs only)	July	Aug.	Oct.	Jan.	Apr.	June
Ampharetidae	A	A	A			A
Sternaspis scutata	B	B	B	B	B	A
Diastylis quadrispinosa	A	A	A		A	A
Periploma fragile			B	B	B	
Harmothoe imbricata			A	A	A	
Rhodine loveni		B	B	B	B	
Nucula tenuis		B	B		B	
Melinna cristata			A	A	A	
Praxillella praetermissa		A	A			A
Unciola irrorata			A		A	
Leptocheirus pinquis		A	A	A		
Diastylis cornuifer		B				
Ninoe nigripes				B		
Micrura spp.					A	
Alvania pelagica			B			
Terebellides stroemii				B		
Diastylis sculpta		A				
Thyasira flexuosa		A				A
Lumbrineris fragilis						A
Astarte borealis			A			
Yoldia sapotilla				B		B
Edotea montosa			A			
Unciola leucopis			A			
Erichthonius difformis					A	
Dentalium entale		B				

scribes differences in benthic species proportions among sampler-types as well as sample size differences (Jolicoeur and Mosimann 1960).

The discriminant analyses were repeated on fishes only (with benthic grab samples removed) to provide a better picture of the degree of feeding niche separation that occurred among fish species. Ninety-five percent confidence elipses on group centroids were plotted (Sokal and Rohlf 1969).

To investigate the robustness of discriminant analysis in analyzing data which violate parametric assumptions, we made analyses on the July site A observations using four types of fish feeding data: (a) nonlogged abundance data; (b) all 217 variables combined into four phyla groups (annelids, arthropods, mollusks, and others); (c) binary data (presence or absence of each variable in a sample); and (d) logged weight data. All data types showed significant and similar group separation on DF I. Gilbert (1968) has found little loss of information when using binary data in discriminant analysis. Discriminant analysis is a good data exploration procedure if the researcher's primary purpose is to explore data shape and structure.

Results

Benthos

From the PONAR grabs taken during this study, 217 invertebrate species were identified. Using the 74 variables chosen by the data reduction technique, a three-way MANOVA showed there to be enough fluctuation in their abundances among times, sampler-types, and between sites to warrant further data exploration ($P < 0.01$).

Benthic species composition differed between sites A and B ($P < 0.01$), DF I; Fig. 3). Site A has large numbers of arthropods (particularly *Diastylis quadrispinosa*) and annelids (*Harmothoe imbricata*, *Melinna cristata*, and ampharetids). Site B had annelids (*Sternaspis scutata* and *Rhodine loveni*) and large numbers of mollusks (particularly *Periploma fragile* and *Nucula tenuis*) ($P < 0.05$; Table 2; Fig. 4 and 5).

Temporal variation of invertebrate abundance and the species composition was greater at site A then at site B ($P < 0.01$; DF II; Fig. 3 and 2). *Leptocheirus pinguis*, *S. scutata*, *N. tenuis*, and the ampharetids were sampled in large numbers in August. *Yoldia sapotilla* was most abundant in June (Table 3). In contrast, *Nephtys incisa*, *Tharynx acutus*, and *Scalibragma inflatum* showed little spatial or temporal variation (Fig. 5). Overall invertebrate abundance was greatest in early fall at site A and early winter at site B. Site B had fewer invertebrates than site A (Fig. 2).

Leptocheirus pinguis sampled from the mud were smallest (0.56−0.76 cm) in summer and largest (1.16 cm) in the fall (two-way ANOVA, $P < 0.05$; Fig. 6). *Casco bigelowi* juveniles appear in the spring (0.57−1.00 cm). However, there was a great deal of size variability among the samples of *C. bigelowi*. *Yoldia sapotilla* were largest in late summer or early fall (0.68 cm) and smallest in early spring (0.17 cm) at both sites ($P < 0.05$). Individuals at site A were larger than those at site B ($P < 0.05$).

Amphipods, ampharetids, and *S. scutata* live at shallower sediment depths than mollusks (*Pitar morrhuana*, *Cylichna alba*, and *P. fragile*) or nemerteans (*Micrura* sp. and *Cere-*

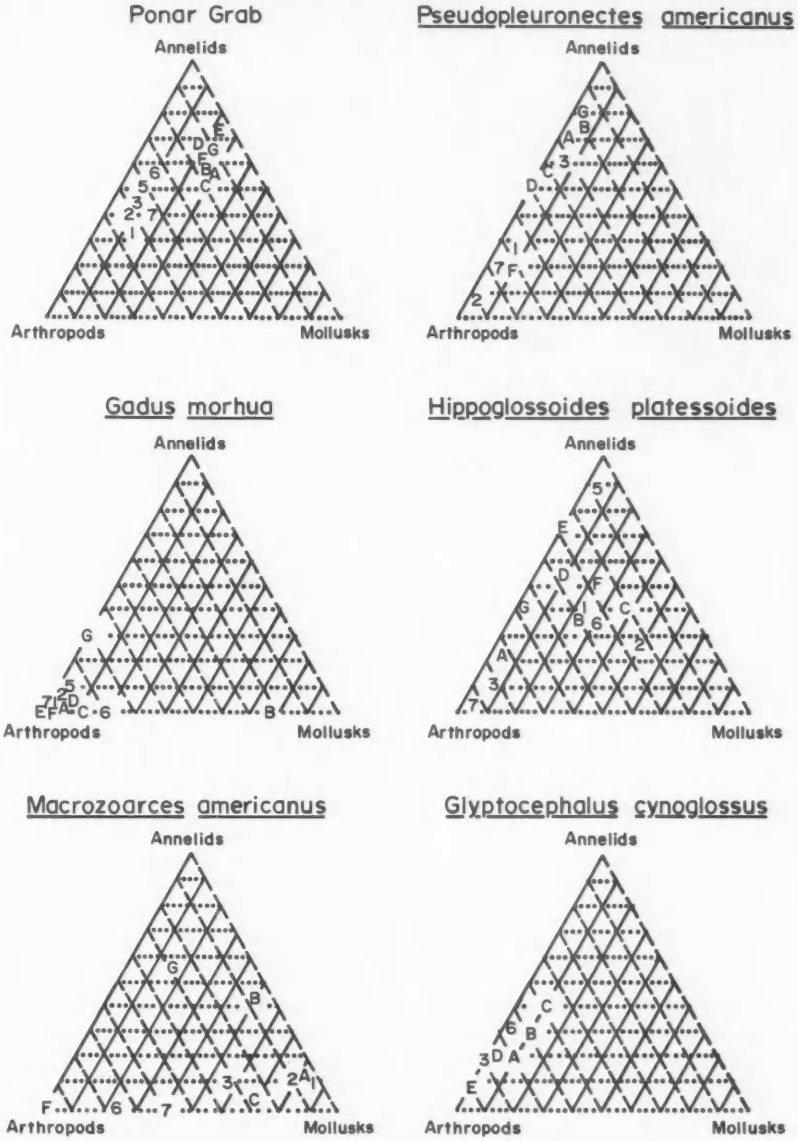
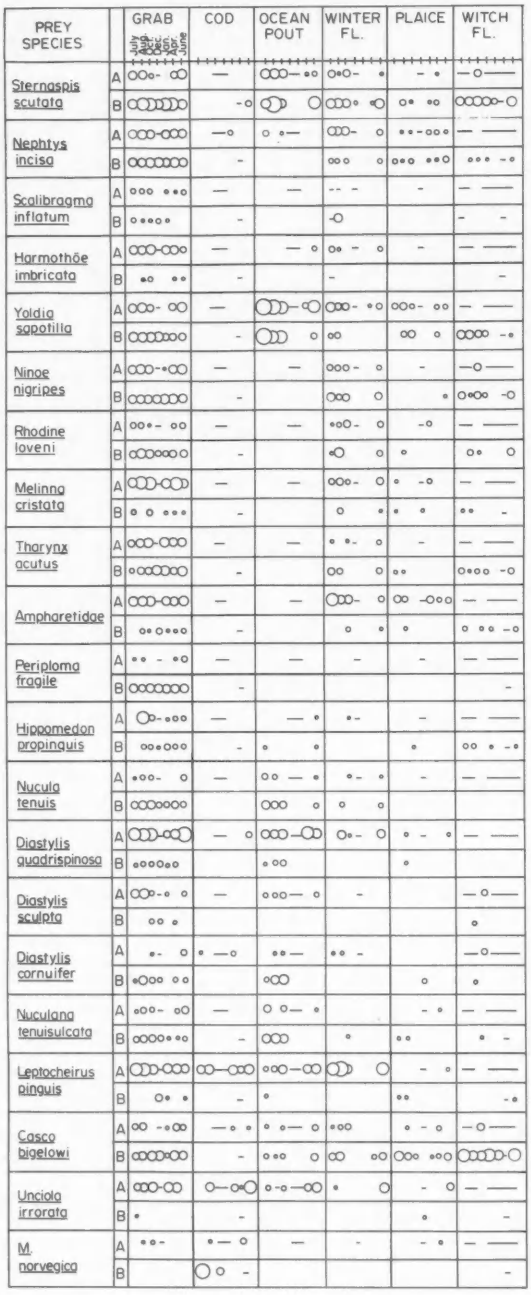


FIG. 4. Percent by number of individuals in phyla Annelida, Arthropoda, and Molluska collected by each sampler-type at each site and time. Broken lines radiating from the lower left corner of each plot each describe 10% decline in Arthropod composition, solid lines radiating from the lower right corner describe Mollusk composition, and dotted lines radiating from the upper corner describe percent Annelid composition decline. Site A collections are shown with numbers and site B with letters. Refer to Fig. 3 caption for more information regarding sampling dates.

bratulus sp.) (ANOVA, $P < 0.05$; Fig. 7). *Yoldia sapotilla* had a shallower mean depth than the other mollusks. The cumaceans sampled were mainly *D. quadrispinosa* and the amphipods were *C. bigelowi* and *Byblis gaimardi*.

Resource Utilization

The predator species fed selectively on the available food resource. The first discriminant function described significant ($P < 0.01$; Fig. 8) separation among benthic species composition and the diets of the five predator species at each site and time. Predators did not utilize all the benthic species that were present in the sediments, nor did they consume prey in the same proportion as the estimated availability. For example, many annelids (*H. imbricata, N. incisa, T. acutus*, and others) are underutilized at most sites and times by most species of fish (Fig. 5). Many mollusks and amphipods are consumed in numbers that are similar to or greater than benthic abundance by some fish species at many sites and times (*Y. sapotilla*,



L. pinguis, *N. tenuis*, and *Diastylis* spp.; Fig. 5).

Cod and plaice consumed fewer prey items during the year and showed less seasonal variation in food consumption habits than the other predators (Fig. 2). Ocean pout and winter flounder ate less food as temperatures declined in the fall. The winter flounder consumed more food at site A than at site B.

There was a great deal of interspecific food resource partitioning. After repeating the series (each site and time) of discriminant analysis without the benthic grab samples, it was clear that the first and often the second discriminant function described differences among the diets of most of the predator species at most of the times ($P < 0.05$; Fig. 9). Winter flounder fed on large numbers of annelids at site B and large numbers of arthropods at site A (Fig. 4). *Praxillella praetermissa*, *H. imbricata*, *Eteone trilineata*, ampharetids, *D. quadrispinosa*, *L. pinguis*, *C. bigelowi*, and *S. scutata* are all consumed by winter flounder in the largest quantities at those times and sites where they are most abundant (Fig. 5). However, despite large quantities at site B, mollusks were only consumed by winter flounder at site A, and then only *Y. sapotilla*, with consumption heaviest in the summer and early fall.

Cod fed on arthropods at most site−time combinations (Fig. 4). They consumed the amphipods *L. pinguis* and *Unicola* spp. (Fig. 5) at site A and mysids and the euphausiid *Meganyctiphanes norvegica* at site B. Unfortunately, mysids and euphausiids are not well sampled with a benthic grab but both types of amphipods were more abundant at site A. *Unciola* spp. were consumed in the largest quantities when they were least abundant in the sediment, during spring. However, the smallest cod were caught in the spring and crustaceans are eaten more frequently by small cod than larger ones (Arntz 1974, 1978a). Annelids and mollusks were consumed in larger numbers in the summer than in the winter.

Plaice are mollusk, annelid, and arthropod feeders throughout the year. Arthropod predation was heaviest at site A (Fig. 4). They utilized *N. incisa*, *S. scutata*, *L. pinguis*, *Eteone rubrocincta*, *C. bigelowi*, *Amphipholis squamata*, and the ampharetids in relative quantities that reflect benthic abundance at most times and sites.

Ocean pout are arthropods predators in April and June (*Unciola* spp., *C. bigelowi*, and *L. pinguis*) and mollusks and annelids predators (*S. scutata*, *C. alba*, *Y. sapotilla*, and *Nucula* spp.) (Fig. 4 and 5) in July, August, and October.

Witch flounder fed on the arthropods *C. bigelowi*, *Hippomedon* sp., and *L. pinguis* and the annelids *S. scutata*, Ampharetidae, and *Ninoe nigripes* at most times of the year in quantities that reflect benthic abundance (Fig. 4 and 5). *Yoldia sapotilla* is the only mollusk consumed in large numbers and is taken more often in the summer.

The smallest amount of resource partitioning, as represented by the overlap in 95% confidence elipses around discriminant score centroids of each predator species (Fig. 9), occurred when feeding rates were low (December, January, and April;

FIG. 5. Densities (mean number of prey) of 20 of the 74 variables (prey species or species groups) of the reduced data matrix in all sampler-types at each site and time. Prey species included are among those which showed significant ($P < 0.05$) abundance differences between sampler-types from Jancey's (1979) ranking program. These are the prey items that the predators prey upon to a greater or lesser degree than expected from their abundance in the sediment. The areas of the circles represent the mean number of prey per fish or grab sample.

TABLE 3. Variables (benthic species) from benthic collections ranked by their ability to discriminate between all pairwise comparisons of successive collection times at each site using the *F*-statistic (Jancey 1979). Variables are significant at the 0.05 level after Bonferroni's inequality equation was applied. Letters in parentheses indicate the month at which each variable was most abundant. Many of the benthic species that show a significant difference in abundance among collection times are listed in Fig. 5.

Time	Site A discriminators		Site B discriminators	
July–Aug.	*Diastylis quadrispinosa*	(J)	*Diastylis cornuifer*	(A)
	Ampharetidae	(J)	*Sternaspis scutata*	(A)
	Praxillella praetermissa	(A)	*Nucula tenuis*	(A)
	Periploma fragile	(J)	*Maldanidae*	(J)
	Diastylis sculpta	(A)	Ampharetidae	(A)
	Lumbrineris fragilis	(A)	*Rhodine loveni*	(A)
	Leptocheirus pinquis	(A)	*Diastylis quadrispinosa*	(A)
	Harmothoe imbricata	(A)	*Nucula dephinodonta*	(A)
			Brada villosa	(A)
Aug.–Oct.	*Edotea montosa*	(O)	*Alvania pelagica*	(O)
	Phyllodoce groenlandica	(O)	*Diastylis cornuifer*	(A)
Oct.–Dec.			*Chaetozone setosa*	(D)
			C. pinnulatum	(O)
Oct.–Dec. (site B)	*Diastylis quadrispinosa*	(O)	*Chaetozone setosa*	(D)
Oct.–Jan. (site A)	*Astarte borealis*	(O)	*Nephtys incisa*	(D)
Jan.–Apr.	*Ninoe nigripes*	(A)	*Terebellides stroemii*	(J)
Apr.–June	*Unciola irrorata*	(A)		
	Yoldia sapotilla	(J)		
	Lumbrineris fragilis	(J)		

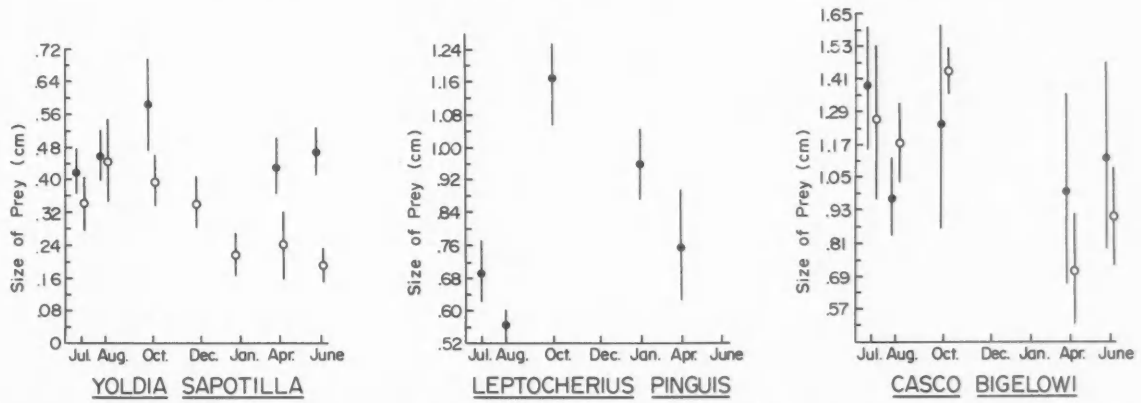


FIG. 6. Mean sizes of three common benthic species collected throughout the year at two sites (only *L. pinguis* from site A were measured). Vertical lines are 95% confidence limits about each mean. Sample sizes: *L. pinguis*, $n = 426$; *Y. sapotilla*, $n = 359$; *C. bigelowi*, $n = 218$. Shaded circles represent ●, site A; ○, site B.

Fig. 2) or during August (site A) and October (site B). In most cases, niche overlap among two or more predators was the result of the sharing of many prey species. This makes it difficult to develop a direct link between benthic change and changes in niche overlap among predator species. For example, winter flounder shared similar abundances of many prey species, with plaice in particular, in August (site A) and October (site B; Fig. 5; *S. scutata*, *Y. sapotilla*, and Ampharetidae). Ocean pout and witch flounder food resources differed from the other predators in August and October because ocean pout consumes mollusks (*Y. sapotilla* and *Nuculana tenuisulcata*) and witch flounder consumes *N. nigripes*, *Y. sapotilla*, and *C. bigelowi*. The similarities between feeding habits of ocean pout and plaice occur in months when they both feed heavily on arthropods (Fig. 4 and 9; October and June, site A) or an arthropod–annelid mix (June, site B). *Unciola* spp., *D. quad-*

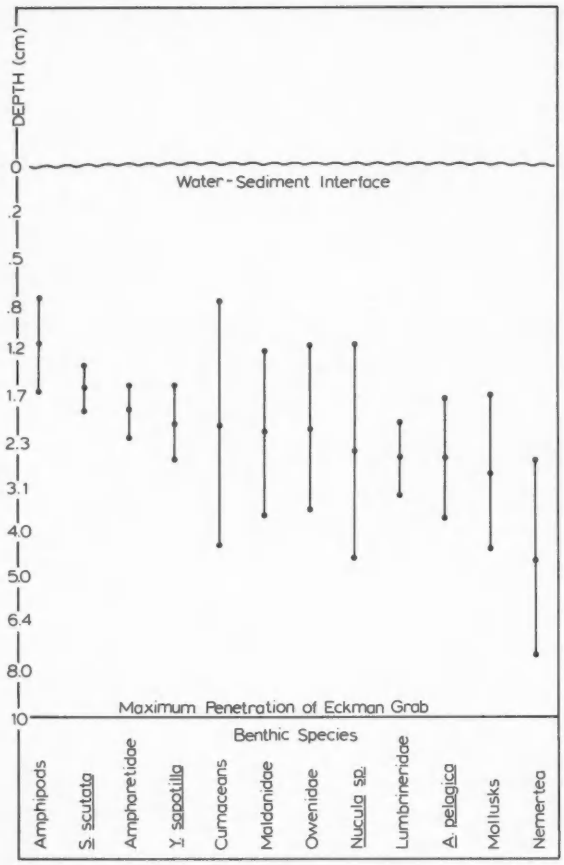


FIG. 7. Mean sediment depths of species of benthic invertebrates ranked in order of descending depth at site A in June. Depths are $\log(X + 1)$ transformed. Vertical bars show 95% confidence limits on benthic species mean depths.

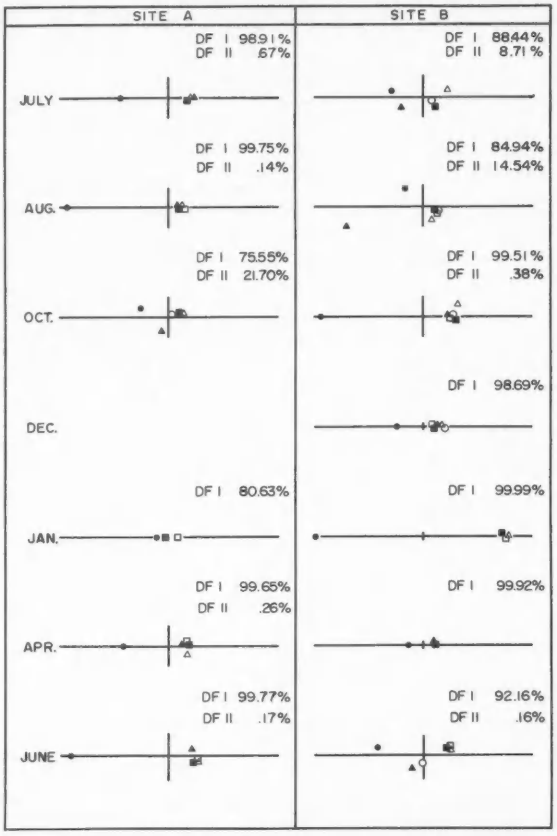


FIG. 8. Results of discriminant analysis on collections of fishes and benthos at each site and time. The percentage of variance explained by each significant discriminant function is presented above each graph. For purposes of comparison the sediment sample centroid is always on the left. ●, ponar grabs; ▲, winter flounder; △, ocean pout; □, cod; ■, plaice; ○, witch flounder.

rispinosa, and *C. bigelowi* were among the prey being shared (Fig. 5). Overlap between the diets of the two predators is much less in July, however, particularly at site B (Fig. 9) because plaice reduce their intake of *Y. sapotilla* and *S. scutata* to consume *C. bigelowi*. At approximately the same time, at both sites, ocean pout switched from arthropods (*Uniciola* spp., *C. bigelowi*, and *L. pingius*) to mollusks and annelids (*S. scutata*, *C. alba*, *Y. sapotilla*, and *Nucula* spp.) (Fig. 4 and 5). Neither of the shifts coincided with changes of benthic prey density in the sediments.

Discussion

Benthos

Many studies of benthic animal abundance at latitudes similar to this study have found annelids to outnumber arthropods or mollusks (Fig. 4) (Richards and Riley 1967; Ziegelmeier 1978). However, crustaceans are more susceptible to losses resulting from sampling device pressure waves and leakage and are therefore underrepresented in most benthic collections (Word 1976).

Temporal benthic species abundance fluctuations can be attributed to the short life spans of many of the benthic species at sites A and B. Many of them spawn in spring and summer (Table 4) (Lacalli 1981) and therefore attain a size that permits them to be caught in large numbers by a 1.0-mm sieve in late summer and fall (Fig. 2). The ampharetids, *Harmothoe* sp., *S. scutata*, most amphipods, and other invertebrates provide examples of this (Fig. 5). *Yoldia sapotilla* and *L. pinguis* are short-lived winter spawners (Table 4) which were also caught most frequently in the summer or fall (Fig. 5). This coincides with the time of year that these invertebrates attained their maximum size (Fig. 6), suggesting that invertebrate size as well as abundance controls the number of animals sampled with benthic grab and sieve. The longer-living invertebrates (*C. bigelowi*) show greater variability in size to each collection time due to the presence of several age classes.

Spatial benthic species abundance differences (sites A and B) likely reflected difference in the degree of environmental stability among the sites (Saunders 1968). Regions with highly variable physical conditions (temperature, salinity, sediment type, current) (site A) (Macdonald et al. 1984) have high faunal diversity (Rhoads et al. 1978) and large seasonal variability in

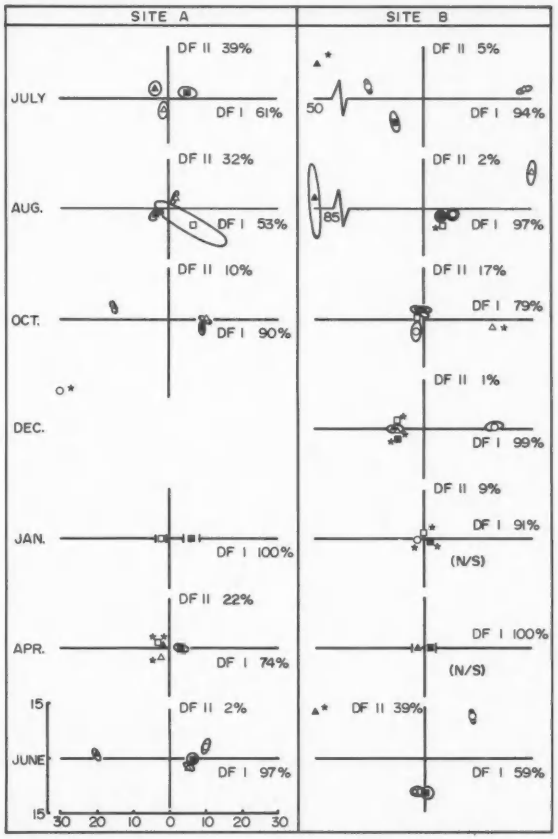


FIG. 9. Results of discriminant analysis on the diets of the five predators at each site and time without benthic grab samples. The percentage of variance explained by each significant discriminant function is presented above each graph. Each centroid is surrounded by a 95% confidence elipse. Asterixes refer to fish species where the variance was too low to calculate ellipses. Low variance may be caused by low sample number or empty stomachs. A discriminant score scale is presented in the lower left hand corner of the figure. Symbols are as defined in the caption to Fig. 8. N/S = no significant difference between the fish.

invertebrate abundance (Rokop 1974). Extremes in temperature (site A) will also reduce the number of bivalves present (Ziegelmeier 1978) (Fig. 5).

Resource Utilization and Partitioning

Demersal fish in many parts of the world consume fewer polychaetes and more amphipods than would be expected from benthic samples (Fig. 4 and 5) (Kiel Bay, Arntz 1978c; Long Island Sound, Richards 1963b; Severn Estuary, Moore and Moore 1976a). However, the researcher's view of what is apparently available may be quite different from what is available to each fish species (Hyatt 1979). Predator species respond in a variety of ways to differences in distribution, escape response, degree of concealment, and size of prey species. The efficiency of the predator's digestion process and feeding methods on each prey type will determine what is observed by the researcher as stomach contents (Steven 1936; Moore and Moore 1976a; Jones 1978; Robb 1981; Macdonald et al. 1982; Macdonald 1983).

Invertebrates that are distributed just below the sediment surface (e.g. amphipods, *S. scutata*, Ampharetidae, and *Y. sapotilla*) are more likely to be consumed by fishes than those that are deeper. Moore and Moore (1976b) speculated that a burrowing polychaete may be difficult for a predator to find and remove. *Periploma fragile*, a mollusk that lives deep in the sediment, occurred in large numbers at site B but was never consumed by fishes. *Yolida sapotilla* lives shallower in the sediment and was consumed in large numbers by most fish species. It was the only mollusk consumed regularly by winter and witch flounder (Fig. 5 and 7). Reading and McGrorty (1978) found the sediment depth of *Macoma balthica* to be the factor that controlled predation rates of wading birds.

Epibenthic invertebrates (mysids, euphausiids, and decapods) use underwater vegetation and other obstacles for protection. Many leave their protected areas only at night, probably timed to avoid the peak feeding activity of visual predators. However, early morning and late evening feeding activity by fish may take advantage of vertical migrations by prey species (Arntz and Brunswig 1975) (Table 4). In the lower Bay of Fundy, nonvisual feeders (ocean pout) may consume large numbers of *Diastylis* spp. at this time (Fig. 5). Alternatively, ocean pout may sort mouthfuls of sediment and thus have access to deeper infaunal material (mostly mollusks) than other predator species (Macdonald 1983).

Visual feeders such as cod and pleuronectids respond to prey movement and therefore feed on epibenthic and shallow infaunal items (e.g. arthropods, Brawn 1969; Jones 1978; Ringler 1979; Libey and Cole 1979; Macdonald 1983) (Fig. 4). Cod and winter flounder eat infaunal material, the cod by digging and the flounder by nipping off siphons or crushing the shells (Bishop and Odense 1966; Frame 1974; Macdonald 1983). The extra time, however, required to search for and handle mollusks makes them suboptimal prey for cod, winter flounder, and probably witch flounder (Macdonald et al. 1982; Macdonald 1983). Plaice have larger mouths which may allow them to consume more mollusks and other calcareous prey than other pleuronectids (Fig. 4 and 5).

Calcareous, and to a lesser extent chitonous, prey are evacuated at a slower rate buy most predators and therefore appear to be eaten in greater numbers than they actually are (Macdonald et al. 1982). Removal of this bias could increase the degree of apparent food niche overlap that exists between the annelid–anthropod predators and the mollusk predators (Fig. 9).

Feeding Reactions to Spatial and Temporal Benthic Change

Temporal, and to a larger extent spatial, changes (Table 2: site A vs. B) in benthic species composition are reflected in spatial and temporal changes in the fishes diets. Many annelids (ampharetids, *P. praetermissa*, and *S. scutata*), arthropods (*C. bigelowi*, *L. pinguis*, *Unciola* spp., and *Diastylis* spp.), and mollusks (*Nucula* spp.) were consumed in greater abundances at the sites and the times that they were more abundant in the sediment (Fig. 5). Demersal fishes do not select and specialize at the species level. Rather, they select food of a general type. For instance, ocean pout eat cumaceans at both sites. At site A they eat *D. quadrispinosa* and *D. sculpta*, the cumaceans that are most abundant at site A. At site B they eat *Diastylis cornuifer*, the cumacean that is most abundant at site B. Winter

TABLE 4. Life histories of benthic species that occurred in large numbers at both sites (C), site B (B), or site A (A). Most information comes from literature sources (Arntz and Brunswig 1975; Barnes 1968) or from personal communications with fellow scientists (D. Wildish and L. Linkletter, St. Andrews Biological Station, N.B.). An "S" marks species that show seasonal fluctuations in abundance. Where available, feeding methods (D, deposit; SU, suspension; C, carnivore), spawning times, life spans (SH, 1−2 yr; L, 2+ yr), and diel vertical migration patterns within the water column (+, migrates; −, does not migrate) are indicated.

Species	Occurrence	Feeding method	Spawning times	Life span	Vertical migrations
Praxillella praetermissa	S−A	D			−
Harmothoe imbricata	A	C	Apr.	SH	+
Eteone trilineata	C	D			
Amphipholis sqamata	C	SU			−
Ampharetidae	A	D	May	SH	−
Melinna cristata	A	D			
Heteromastus filiformis	B	D	Apr.−May		−
Rhodine loveni	B	D			−
Chaetozone setosa	B	D			−
Sternaspis scutata	S−B	D	Sept.		−
Cerastoderma pinnulatum	S−A	SU			−
Nucula sp.	S	D			−
Nuculana tenuisulcata	B	D			−
Astarte sp.	A	SU		L	−
Periploma fragile	B	SU			−
Leptocheirus pinguis	S−A	SU	Jan. or July	SH	
Diastylis sp.	A	D		SH	+
Unciola sp.	A	D		SH	
Casco bigelowi	S−B	D	July	L	
Arctica islandica	S−C	SU			−
Syrrhoe crenulata	S−C	D		SH	
Nephtys incisa	C	D/C	May	L	
Lumbrineris fragilis	C	C	July−Aug.		−
Scalebragma inflatum	C	D			
Yoldia sapotilla	S−C	D	Winter		−
Harpina propinqua	C	D		SH	
Thyasira flexuosa	S−C	SU			−
Haploops fundiensis	S−C	SU		SH	
Orchomonella minuata	C	D		SH	
Ninoe nigripes	S−C	D	Sept.		−
Owenia fusiformis	S−C	SU/D		SH	−

flounder eat amphipods at both sites. At site A they consume the dominant *L. pinguis* and at site B the dominant *C. bigelowi*.

Changes in size of benthic animals can also be responsible for dietary shifts (Pearcy 1962; Levings 1974; Moore and Moore 1976a; Arntz 1974; 1978a). *Leptocheirus pinguis* is more heavily utilized by many fishes when it is largest, from October to April. *Yoldia sapotilla* is more heavily utilized by winter and witch flounder and ocean pout when it is largest in the summer. *Casco bigelowi* is more heavily utilized in the summer, when it is largest, by plaice and winter flounder (Fig. 5 and 6). Dietary shifts towards these prey species are not caused by increases in their benthic density. In fact, *Y. sapotilla* and *L. pinguis* are more abundant during the times of year when predation pressure is minimal (therefore, demersal fishes may play a role in controlling population levels of a few common species (Levings 1974). However, predation impact on most benthic species is negligible. Richards (1963a), Arntz (1978b), and Choat (1982) reached similar conclusions). According to optimal foraging theory (MacArthur and Pianka 1966) the predator will ignore small prey where the yield in energy per unit foraging time is sufficiently lower than that of larger prey. Since energy content usually increases with body size within a prey species (Kerr 1979), there exists a prey size where the ratio of energy gained to time spent foraging is maximized (MacArthur and Pianka 1966; Krebs 1978). Larger prey will also be more visible.

In the lower Bay of Fundy region, as at other locations (Sedberry and Musick 1978; Pearcy and Hancock 1978; Ringler 1979; MacPherson 1981), demersal fishes showed the greatest diet overlap when benthic density was highest (Fig. 2 and 8), in late summer − early fall. Other factors, such as daily feeding times, spatial and temporal variation in predator abundance, and vertical differences in areas of feeding activity (Johansen and Ringler 1980), may also serve to reduce niche overlap, particularly among winter flounder, plaice, and cod in August (site A) and October (site B). However, it is likely that food type is the major niche segregating factor (Gascon and Leggett 1977; Keast 1978; Kravitz et al. 1976; MacPerson 1981).

We do imply that fish "prefer" or "select" specific prey in the deliberate sense of the words. Rather, food specialization and dietary niche separation are a result of evolutionary development of unique feeding behaviors, digestion morphologies and techniques, and mouth structures which interact with the

size, behavior, distribution, and abundance characteristics of certain types of available benthic fauna. For instance, the development of techniques to efficiently feed and process infaunal food partially explains the predominance of mollusks and sedentary polychaetes in the diet of the ocean pout (Macdonald et al. 1982; Macdonald 1983). Visual feeders (winter flounder, cod, plaice, and witch flounder) are more likely to consume epibenthic prey. As a result, many demersal fish species segregate the resource by having somewhat specialized food habits. However, they must also cope with spatially and temporally changing food resources and therefore specialize in general prey types, not prey species.

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Genetic, Environmental, and Maturational Effects on Atlantic Salmon (*Salmo salar*) Survival in Acute Low pH Trials

C. B. Schom[1]

Department of Biology, University of New Brunswick in Saint John, and Atlantic Salmon Federation, P.O. Box 429, St. Andrews, N.B., E0G 2X0

Schom, C. B. 1986. Genetic, environmental, and maturational effects on Atlantic salmon (*Salmo salar*) survival in acute low pH trials. Can. J. Fish. Aquat. Sci. 43: 1547–1555.

Wild sea-run anadromous salmon (*Salmo salar*) were captured in Big Salmon River in New Brunswick and transported to the Atlantic Salmon Federation's research centre for spawning. Four year-classes were established, one each in 1980, 1981, 1982, and 1983, using a nested design, i.e. one female with one anadromous male and one or two mature parr. The mature parr, in all but the first year, were from families identified as to survival time in acute low pH trials. Resistance to low pH, i.e. survival time, was family specific and independent of the trial conditions. The heritabilities had dominant and additive components with, however, the apparent larger dam than sire effect an artifact of the experimental design. The most significant component appeared to be the dominant gene component. The mature parr were more resistant than the nonmature parr. Finally, genetic gains from selection were much greater in the down direction than the up direction, and this appeared to be a consequence of negative hatchery selection.

Les saumons sauvages anadromes de mer (*Salmo salar*) ont été capturés dans la rivière Big Salmon au Nouveau-Brunswick et transportés au centre de recherches de la Fédération atlantique du saumon pour frayer. On y établit quatre classes d'un an, chacune pour 1980, 1981, 1982 et 1983 selon un concept de nidification, c'est-à-dire une femelle avec un mâle anadrome et un ou deux tacons à maturité. Les tacons à maturité, dans tous les cas sauf la première année, venaient de familles reconnues pour leur durée de survie dans des essais d'abaissement aigu du pH. La résistance à un pH abaissé, c'est-à-dire la durée de survie, était une propriété spécifiquement familiale et indépendante des conditions de l'expérience. Les caractères héréditaires comportaient des composantes dominantes et ajoutées bien que l'effet apparent plus prononcé de la mère que du père relevât d'un artifact du schème expérimental. La composante la plus importante semble être celle du gène dominant. Les tacons à maturité ont montré plus de résistance que leurs cadets. Enfin, les gains génétiques de la sélection ont été plus marqués vers le bas que vers le haut — ce qui semblerait découler de la sélection négative pratiquée dans l'établissement de pisciculture.

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Surface water acidification has been implicated in the decline and/or elimination of several fish species, including salmonids, in North America (Beamish and Harvey 1972; Beamish 1974, 1976; Thompson et al. 1980) and Scandinavia (Almer et al. 1974; Leivestad and Muniz 1976). The critical pH below which no Atlantic salmon (*Salmo salar*) recruitment occurs was reported for Nova Scotia rivers to be between 4.7 and 5.0 (Watt et al. 1983) and for Swedish rivers to be between 5.0 and 5.5 (Jensen and Snedvik 1972). Kerekes (1983) maintained that Watt et al.'s (1983) estimated lower critical pH for the Nova Scotia system occurred because no correction was made for organic anion. If Kerekes (1983) is in error, then the difference in critical pH between European and North American salmon may be genetic, as has been indicated by Schom et al. (1984) for other traits.

Strain and stock specific differences in low pH resistance have been reported for several species, including brook trout (*Salvelinus fontinalis*) (Swartz et al. 1978), yellow perch (*Perca flavescens*) (Rahel 1983), and North American Atlantic salmon (Schom 1985). Gjedrem (1976) for brown trout (*Salmo trutta*), Swartz et al. (1978) for brook trout, Rahel (1983) for yellow perch, and Schom and Davidson (1982) for Atlantic salmon reported genetic variations which give heritability estimates large enough to make selection for low pH resistance a theoretical possibility. This would be contrary to Gorham (1981) who argued that genetic modification will not succeed in keeping stocks in low pH river systems.

My objective was first to confirm that genetic control existed and then to compare the theoretical estimates with the realized estimates. Finally, I attempt to explain just what characteristics of the individual were correlated with survival time and/or altered the accuracy of the estimate, therefore reducing the correspondence between realized and theoretical estimates.

Materials and Methods

The approach was to repeat the experimental design in each of four years, 1980–83, with minor modifications as a way

[1]Present address: Aitken Bicentennial Exhibition Centre, 20 Hazen Avenue, Saint John, N.B. E2L 3G8.

TABLE 1. Summary of experimental design conditions, number of matings, and number of fish challenged in each of the trials.

Year spawned	Experimental design	Number wild anadromous		Number mature parr	Number spawn dates	Trial	
		Female	Male			Months post-hatch	Number fish challenged
1980	Nested 1:2	9	9	9	2	1	5268
						8	109
						9	121
1981	Nested 1:3	13	13	26	3	4	8156
						6	2251
1982	Factorial 3:4	3	3	9	1	5	2929
						8	4800
	Nested 1:3	9	9	18	1	6	1866
						8	1800
1983	Nested 1:3	2[a]	3	9	1	10	1339

[a]Plus one cage-reared anadromous female from a known family.

TABLE 2. Correlation between family ranks for different trials within a year-class. Included are some of the environmental conditions associated with each trial. *Statistically significant at the 5% level (regression analysis); **statistically significant at the 1% level (Spearman rank correlation analysis).

Year spawned	Correlation of family rank between repeat trials in the same year	Trial 1				Trial 2			
		Months post-hatch	Mean temp. (°C)	Mean pH	Mean survival time (h)	Months post-hatch	Mean temp. (°C)	Mean pH	Mean survival time (h)
1980	0.80*	1	13.5	4.3	131.3	10	4.0	3.2	36.1
1981	0.61**	4	17.0	4.2	234.3	6	13.5	4.0	33.6
1982	0.71**	5	15.5	4.0	92.8	8	6.8	4.0	370.3

of compensating for the small number of families the facility could accommodate. The parameters were estimated applying all techniques to each year-class. Thus, consistency between year-class results compensated for large standard errors and can be interpreted to mean that the estimates reflect what existed in the population as a whole.

All stock was originally from a random sample of late-run anadromous fish returning to the Big Salmon River, New Brunswick. With the exception of one fish used in 1983, all males were either wild sea-run anadromous returns to the Big Salmon River or mature parr from the Big Salmon River stock reared at the Atlantic Salmon Federation's research facilities for one or more generations. In 1980, the parr sires were a random sample taken from a randomly chosen tank which held fish from crosses made at the Atlantic Salmon Federation's research centre the previous year. In subsequent years, they were pedigreed and selected for either low or high pH resistance sires.

Mature parr were used because (1) they appear to both sire offspring in nature (Saunders and Schom 1985) and (2) artificial selection can be applied to the male line within the first year rather than after the 3 or more yr necessary to wait for sea-run fish to mature. Two assumptions were made. The first was that no correlation existed between age of maturation and the survival time in low pH, the trait of interest. The second was that the anadromous male crossed to the anadromous female provided a random sample of genotypes from the Big Salmon River and therefore acted as an unselected control against which the selected families could be compared.

The experimental design used in all but one trial nested two or three males within a female, that is each female's eggs were split into two or three lots with a different male used to fertilize each lot. For details and genetic consequence of these procedures, see Becker (1968), Falconer (1981), and/or Van Valeck (1982). The exception in 1982 had both a nested component and three sets of a 3 × 4 factorial including as sires in each of the sets a pair of full sibs. One sib had survived the low pH challenge in 1981; the other was randomly selected from among mature parr in that family. They differed, therefore, in a second way from other mature parr used in this cross, as they were in their second year after hatching (P1+) rather than their first year after hatching (P0+). For more detail of the experimental design, see Table 1.

With the exception of the first low pH challenge in 1980, all individuals to be tested were hot branded and randomized to one of four communal holding tanks at least 2 wk before test. The challenge was run in a recirculation system using four tanks 1.1 m in diameter with a flowrate of about 3 L/min and 10% replacement/h. A radiometer end point titration system, monitoring and feeding sulfuric acid into the reservoir tank, was used to regulate pH at the lower end of, or below, Wood and McDonald's (1982) ecologically significant level. The water calcium concentration was 3.4 ppm, and the conductivity was 34.2 μmho/cm. No feeding was allowed during the trial to minimize nitrogen buildup. When checked, the nitrogen level did not exceed normal lake levels.

The one exception in 1980, i.e. no fish branded because they were too small, used 1-mo post-hatch fry challenged in the

TABLE 3. Heritability of survival time in each year-class calculated using the nested analysis of variance at the indicated age post-hatch.

Brood year	Months post-hatch	$h_d^2 \pm$ SE	$h_s^2 \pm$ SE	$h_{s+d}^2 \pm$ SE
1980	1	0.54±0.30	0.18±0.08	0.36±0.16
1981	4	0.62±0.26	0.19±0.06	0.40±0.14
1982	5	0.57±0.30	0.22±0.09	0.39±0.16

TABLE 4. Heritabilities calculated in two ways: as a sire–offspring regression and as a realized heritability based on the difference between the wild anadromous by wild anadromous control and the positively selected sire families or to the negatively selected sire families.

Brood year	Offspring regression	Realized[b]	
		Up ± SE	Down ± SE
1980–81	0.26±0.76[a]	0.12±0.11	0.23±0.07
1981–82	0.36±0.26[a]	0.07±0.28	0.32±0.36

[a]Standard error of the estimated heritability.
[b]SE calculated following the procedures of Prout (1962).

TABLE 5. Comparison of the mean performance of offspring from a cross using a mature parr sire that had survived a low pH challenge with the performance of the offspring from a randomly selected unchallenged full sib.

Sire family	Mean survival time	
	Acid survivor	Sib's family
1	102.1	90.9
2	92.6	80.8
3	90.5	84.7

above described recirculation system. Each family was held in a 0.3-m container, four containers to a 1.1-m tank. The pH was adjusted by hand twice daily.

The experimental objective was to challenge the fish for 10 000 min (2 wk) or until all of the fish were dead. In several tests, the environmental conditions were modified so that death occurred far more rapidly. In others, the experiments were terminated before all of the individuals were dead (Table 2). The distribution of the variable survival time was obtained by removing and identifying the dead fish every 3 h. The brand, time surviving on trial, length, etc., were recorded.

The operculum erosion was indexed in the 10-mo post-hatch trial using the 1983 year-class fish. The operculum was scored as 0 (no damage), 0.5 (moderate damage), or 1 (severe damage) for each side and then summed for the two sides to establish the index score.

All unwanted variables showing a significant correlation were corrected using linear regression. This was the procedure used by Schom (1985), Hurley and Schom (1984), and others. All effects were assumed to be fixed and all measurements were assumed to be taken with minimum error; therefore, little correlation existed between regression coefficients. The philosophy applied was to use the procedure which gave the approximate results rather than the most mathematically elegant approach. The largest source of error was assumed to be the small number of families, and this could not be corrected by any statistical sophistication. To summarize, a least-squares step-down regression analysis was applied to all nongenetic independent sources of variability. Corrections were then made for effects that were significant. For the mathematical models used to make the corrections, see the Appendix.

The hierarchical analysis of variance was used to partition the sums of squares in the nested designs. The reduced random effects model adjusted for fixed effects was

$$y_{ijk} = u + D_i + S_{ij} + e_{ijk}$$

where y_{ijk} is the individual measurement, u is the population mean, D_i is the random effect of the ith dam, S_{ij} is the random effect of the ijth sire nested in the ith dam, and e_{ijk} is the random error among fish in an individual family.

The heritabilities, their standard errors, and genetic, environmental, and phenotypic correlations were calculated following the procedures of Becker (1968).

The two-way analysis of variance was used to partition the sum of squares in the factorial design. Linear regression was used to remove the nongenetic effect associated with set (Appendix). The reduced random effects model adjusted for fixed effects was

$$y_{ijk} = u + D_i + S_j + (DS)_{ij} + e_{ijk}$$

where y_{ijk} is the individual measured, u is the population mean, D_i is the random effect of the ith dam crossed with jth sire, S_j is the random effect of the jth sire crossed with the ith dam, $(DS)_{ij}$ is the interaction of the ith dam mated to the jth sire, and e_{ijk} is the random error among fish in an individual family.

The heritability estimate and average degree of dominance followed the procedures of Comstock and Robinson (1949) with the exception that there were no replications or blocking in the experimental design. The sire–offspring and realized heritability followed the procedures of Falconer (1981). All other statistical procedures followed those of Snedecor and Cochran (1968), Duncan (1967), Guenther (1964), or as part of the Statistical Analysis System software package on the UNB IBM computer (Anonymous 1979).

Results

The correlations of survival time (ST) family rank between trials within year-class were statistically significant (range 0.61–0.80) for very different sets of conditions (Table 2). The same was true for the sire's own family rank in trials for its year-class and the rank of its offspring family in the next year-class (rank 0 79, 1980 sire year with 1981 offspring year, and rank 0.89, 1981 sire year with 1982 offspring year, significant at the 1 and 3% level, respectively). This was true even though the uncorrected mean length of time families survive low pH challenges was highly dependent on the trial conditions. For examples of variability in family means, STs, etc., in other trials, see Schom (1985).

The heritability estimates for ST were relatively constant for fish aged 1–5 mo, with the dam estimate about three times larger than the sire estimate (Table 3). No estimates were reported after 6 mo of age because a major source of variability which first appeared at 6 mo was not identified until 1984. No

TABLE 6. Correlation of survival time rank with livability rank in the hatchery for all families challenged and for the one third of families with the best survival time. *Statistically significant at the 5% level; **statistically significant at the 1% level.

Year	Time for which survival in hatchery (livability) estimated	Number of families	Correlation coefficient for survival time with livability (all families)	Correlation coefficient for survival time with livability (highest 1/3 survival times)
1981	From branding to trial[a]	39	0.65**	−0.7**
1982	From branding to trial[a]	12	0.53*	−0.8*
	From branding to trial[a]	20	0.51*	−0.5
	From branding to trial[a]	16	0.33	−0.8*
	From eyed egg to transfer	16	0.56*	−0.5

[a]Hatchery survival (livability) calculated for families in communal tanks, i.e. all families were together in a common tank.

TABLE 7. Heritability of length calculated from the nested analysis of variance for the year-classes and ages indicated.

Brood year	Estimate	Months post-hatch	$h_d^2 \pm$ SE	$h_s^2 \pm$ SE	$h_{s+d}^2 \pm$ SE
1981	1	4	0.72±0.33	0.36±0.10	0.53±0.18
	2	6	0.29±0.18	0.40±0.13	0.35±0.13
1982		5	0.39±0.78	0.53±0.19	0.46±0.19

TABLE 8. Genetic, environmental, and phenotypic correlation of length with survival time calculated for the year-classes and ages indicated.

Age brood year	Post-hatch (mo)	r_{gd}	r_{gs}	r_{gs+d}	r_{ed}	r_{es}	r_{es+d}	r_p
1981	4	0.35	0.25	0.32	0.20	0.27	0.29	0.27
1982	5	−0.46	0.23	−0.16	0.38	0.10	−0.02	0.06

direct measurements were made of this parameter in the earlier years. No correction could, therefore, be made; thus, heritability estimates for fish in the older age-class were meaningless.

The sire−offspring regression h^2 estimates (Table 4) were about the same size as the sire−dam h^2 estimates within a year-class (Table 3), both being about 35%, but with very large standard errors. This is in contradistinction to the realized heritability which exists but was very different in the positive and negative direction: 0.07−0.12 and 0.23−0.32, respectively (Table 4). The positive selection for ST was probably zero because the standard errors were very large. In fact, the hatchery environment seemed to apply pressure against increased ST (see later section for details). Overall, however, the realized heritabilities correspond to the sire estimate generated from the factorial analysis of variance which was 0.14 and those calculated using the nested analysis (Table 3). The estimates of dominance deviations were 2.5 times larger from the dam components at 4.4 than from the sire at 1.67.

Two sires in each set from the factorial design were full sibs, one an individual that survived a low pH trial and the other a randomly selected individual from the same family. In all instances, the mean ST for the families of the sire that survived the acid trial was greater than for its full sib's families (Table 5).

In a year-class, the family ST rank was positively correlated with the rank of the family's survival in the hatchery. This does not tell the whole story, as the correlation between ST and livability for the one third of the families with the longest ST was significant and negative (Table 6). Livability was, in all but one instance, calculated for families identified and held in a communal tank.

Heritability of body length was fairly constant with one exception, and that one had a very large standard error (Table 7). The variability between year-classes was large in the estimates of genetic, environmental, and phenotypic correlations made between ST and length (Table 8).

While statistically significant phenotypic correlations existed between length and ST when all individuals were pooled in a trial, the number of families with significant correlations varied from trial to trial. In general, 70% or more of the families at 4 and 5 mo post-hatch had a significant correlation (Table 9), as did 10-mo post-hatch fish (Table 10). The proportion was lower in the 6th mo.

There was very little difference in mean length between those families having a correlation between ST and length and those having no correlation. However, there was a difference in the proportion of the parr that matured in a family. The families that showed a significant positive correlation between ST and length had, on average, fewer parr maturing (Table 9).

In the 1983 year-class, the proportion of mature parr identified as mature by squeezing semen from them ranged from a low of 9% to a high of 37% within families. In all but one family, the mature individuals survived significantly longer than the immature individuals (Table 10) even though their mean length was shorter (Table 11). The opercular condition was statistically significantly better in only 2 of the 6 families and significantly related to length in only 3 of the families, and then only in the set that were not mature (Table 11).

When all of the families were pooled, the relative proportion of mature parr dying in each 24-h period increased from 0 to

TABLE 9. Comparison of families having a statistically significant correlation between length of fish and survival time with families showing no correlation. There was no difference in mean survival time or length of fish between the two groups.

			Correlation, length with survival time			
			Significant (1% level)		Not significant	
Year	Age (mo)	Total number of families on trial	Number of families	Mean percent mature parr	Number of families	Mean percent mature parr
1981	4	38	34	8.6	4	14.5
	6	34	5	5.2	31	9.5
1982	5	26	19	5.9	7	8.8
	6	14	2	1.5	12	8.3

TABLE 10. Comparison of mean survival time generated in an acute pH challenge for mature and immature individuals. The dam and sire numbers identify individual parents. The offspring were from the 1983 year-class and 10 mo post-hatch when challenged. *Significantly different in a family at the 5% level (t-test); **significantly different in a family at the 1% level (t-test).

				Mean survival time		
Dam	Sire	Number challenged	Percent mature	All	Mature	Not mature
7	0	135	9	139	177*	135*
	2	20	30	155	183	143
9	2	384	24	82	93**	77**
	3	368	19	78	106**	72**
8	0	98	36	156	190**	137**
	3	339	37	146	185**	124**

73% as the experiment progressed. There were only two drops, one from 34 to 28%, and this after a downward fluctuation in pH. The second drop from 50 to 42% occurred after all of the members of two families had died (Fig. 1).

Discussion

Genetic Components

Atlantic salmon seem to have genetic variation for survival in acute low pH. Given a uniform set of trial conditions, the ST rank of the families in one trial did not change markedly in the next trial, even though trial conditions were different (Table 2). Also, the sire's own family rank corresponded to its offspring family rank. In addition, offspring of sires surviving a low pH challenge outperformed nonchallenged sib's offspring (Table 5). Finally, Schom (1985) reported that those fish coming from river systems with a history of deteriorating pH outperformed, in acute trials, those fish from more pristine systems. By implication, then, the results reported here for acute conditions should apply to responses under chronic conditions and vice versa. If the foregoing is true, then the 30% survivors reported by Lacroix et al. (1985) in his pH 5.0 challenges would be individuals carrying genes for increased survival not only in his chronic conditions, but also for acute conditions. This question deserves further study.

The h^2 estimates for resistance to low pH are moderate and correspond to those reported for other species (Gjedrem 1976; Swartz et al. 1978; Rahel 1983), but differ depending on the estimation procedure. The sire estimates which were biased by selection in 1981 and 1982 (Tables 4 and 5), the sire–offspring regression which was not biased by selection, and the realized heritability (Table 4) were all about the same size. The dam estimated (Table 3) was very different and larger. This is troublesome because the experimental design used — sires nested within dams — should give a dam estimate with all but some interactions and the maternal effect removed (Van Veleck 1982). Therefore, either the maternal effect was very large, which is unlikely, or something else occurred.

Maternal effects tend to decline as the offspring age. Here, there was, in general, no decline in several years of data in the size of the effect causing the higher dam estimate than sire estimate until a shift at the 6th mo. The shift was so large that the estimate seemed meaningless and therefore was not reported. The cause of the shift will be discussed later.

A genetic explanation requires that the genetic variability among sires be smaller then among dams. This was likely so, resulting first from an error in assumption and second as a consequence of the experimental design. The assumption in error was that parr maturation was a random event; therefore, if families were sampled at random as they were in 1980, a random set of parr sires would be selected. The fact is that most families have few mature parr and a few families have a lot of mature parr (unpublished data). The mature parr collected were likely from the one or two families with the high incidence of maturation. That is, while there were twice as many sires as dams in 1980, they represented only a few families, giving a low effective number, thereby a low overall sire genetic variance. Attempts were made to rectify this when parr sires were selected for the 1981 and latter year-classes by choosing some wild by wild sired mature parr, but the very fact that the parr were chosen meant that the sire set was nonrandom and probably more uniform than the random set of dams. This no doubt meant that the effective number of parents on the male side remained smaller than was originally thought.

In 1982, the genetic variance among sires used in the factoral analysis was smaller than among dams, not only for the reason given above, but also because the two sibs (brothers) were included as sires in each set of crosses. This was done recognizing the cost in reduced genetic variance but seemed the best way of including crosses, comparing offspring responses of sires surviving a low pH challenge with those of unchallenged sibs. This no doubt helps to explains the lower dominance deviation estimate and small heritability estimated from the sire

TABLE 11. Comparison of mean length and opercular condition for mature and immature individuals evaluated in an acute pH challenge. The dam and sire numbers identify individual parents. The opercula were scored as 0 (no damage), 0.5 (moderate damage), or 1 (severe damage) and then summed for the two sides to establish the score. The trial was run using 10-mo post-hatch 1983 year-class parr. **Significantly different at the 1% level (t-test).

Dam	Sire	Number challenged	Mean length		Mean opercular condition		Significant level for correlation, opercular condition with length of fish	
			Mature	Immature	Mature	Immature	Mature	Immature
7	0	135	8.8**	10.5**	0.4	0.2	0.070	0.71
	2	20	10.6	11.2	0.1	0.4	0.24	0.10
9	2	384	8.5**	9.2**	0.3**	0.5**	0.91	0.93
	3	368	8.3	8.3	0.9**	1.0**	0.74	0.002
8	0	98	9.5**	10.9**	0.1	0.2	0.73	0.002
	3	339	9.8**	10.5**	0.6	0.6	0.07	0.002

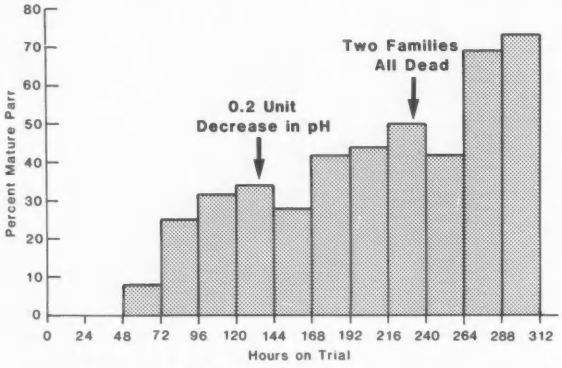

FIG. 1. Percentage dead parr that were mature calculated for each 24-h period plotted against the number of hours on experiment. The trial was run using 10-mo post-hatch 1983 year-class fish, i.e. in January 1985.

components in the factoral experiment.

On their own, the estimated dominance deviations are very weak evidence for the effect, in particular because there was no replication or blocking in the experimental design. Thus, the estimates include nongenetic sources of error. In each of the years, however, the results from the nested design give support and were, in fact, the reason for attempting to generate an estimate, even one as weak as this. The dam families with the higher or lower ST tended to group together near the top or bottom of the ranking. It appeared as if the upper range offspring inherited genes from the dam with dominance effects for increased survival, as all her families ranked near each other. The lower range offspring appeared to inherit genes with dominance effects for reduced survival. Those in the middle seemed to be missing these genes from the dams and responded to the components they received from the male. This seemed to be true from 1 to 10 mo post-hatch, i.e. long after material effects should have been lost. The results, therefore, from the factorial analysis and the observations from the nested analysis support each other, indicating that effects of dominance were present. Furthermore, the sire h^2, while smaller than the dam h^2, also had smaller standard errors (Table 3) and therefore appeared to be significant. This likely reflected the inclusion of a portion of the dominance effect (Van Veleck 1982) in sire estimate, reducing the variability within a sire family, offering yet another explanation for the apparent greater genetic similarity between sires.

The size of the dominance deviation, while smaller from the sire estimate, is still large enough to imply that dominant gene effects play a major role in the control and thus transmission of resistance from generation to generation. Therefore, quantitative genetic experiments should be designed to evaluate, in more detail, the effect of dominance. Finally, if dominance deviations are as large as implied here, before artificial selection can act on the additive components, these major gene components are going to have to be fixed or eliminated.

Hatchery Selection

The realized heritability was different for the upward and downward selected families and may have resulted from artificial selection working against hatchery selection. Over all families in a trial, there was a statistically significant positive correlation between survival in the hatchery and ST in low pH challenge. This was not true for the one third of the families with the best ST. These families had statistically significant negative correlations between ST and livability (Table 6). In fact, a number of the families with the best ST did not have any offspring alive in the hatchery to use as sires in the next generation. The combination of genes that increased ST in low pH trials appeared to reduce survival in the hatchery. These results are difficult to dismiss as a sampling error because they occurred 3 consecutive yr with different sets of families in each year. In addition, almost all of the estimates for livability were made on fish held in a common, communal tank. The loss of specific families, presumably with particular gene sets, provides an explanation, other than population bottleneck, for the origin of Stahl's (1983) genetic difference between hatchery and wild stocks.

Mature Parr

Mature parr survived a longer time on trial than their nonmature sibs. At 4 mo post-hatch, many more families had a significant positive correlation between ST and length than at 5 or 6 mo (Table 9) which was the time when Thorpe et al. (1980) reported that bimodality first began to appear, and by implication, when the parr begin to mature. Bailey et al. (1980) reported that most of the mature parr were found in the lower mode. Therefore, maturation which probably slows growth also appears to alter the length−ST relationship.

Thus, the onset of maturation of 6 mo was likely the source

of the unidentified variability that made heritability estimates unrealistic for that age group (see earlier and Appendix). This apparent maturation effect seemed to increase the variability within the family but did not alter the relative rank of the family. The effect was to increase the ST of maturing parr, and thus extend the upper range of ST with small fish if the 10-mo explanation can be applied to the 6-mo post-hatch results. The effect of maturation should be examined in detail.

The increased survival is a little difficult to explain, as increased steroid hormone levels tend to hamper ion retention (Mazeaud and Mazeaud 1981). Even though the skin is thicker in mature parr than immature parr (Murphy 1980), little or no ion or water exchange takes place across the skin (Anderson and Mitchum 1974). No other trait identified by this author seemed to be correlated with the increased survival. But mature parr did out-perform immature parr of the same age, and therefore, an explanation is needed.

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Appendix

The mathematical models listed in Table A.1 are the ones used to correct for fixed effects in each of the years and trials. While some of the effects may be confounded, for example incubation effect measured as spawn date and rearing effect measured as hatch date, it is the author's view that they identify separate effects, as they are handles that identify a whole series of effects the group spawned together experienced during incubation, including temperature regimes, type of incubation, etc. In the same way, hatch day is an identifier for a number of

environmental effects, including a common rearing-tank effect, etc. Great care was taken with the 1982 year-class to avoid both spawn day and hatch day differences so that the analysis was much cleaner, requiring correction for only one factor. It must also be kept in mind that this procedure can create correlations among the adjusted observations, and in addition, the effects may not be linear. When checked, the assumption of no correlation and linearity appeared to hold, at least within the range of values in the data sets.

TABLE A.1. Mathematical models.

Year	Months past post-hatch	Mathematical model
1980	1	$Y_{ijk} = y_{ijk} - b_i(D_i - D.) - b_j(T_j - T.) - b_k(P_k - P.)$
		where Y_i is the survival time corrected for the ith incubation effect, jth temperature during challenge, and kth pH
		y_{ijk} is the survival time measured for the ith incubation effect, jth temperature during challenge, and kth pH
		b_{i-k} is the regression coefficient for the ith incubation effect, jth temperature during challenge, or kth pH
		D_i is the ith incubation effect
		T_j is the jth temperature during challenge
		P_k is the kth pH
		$D.$ is the mean incubation effect
		$T.$ is the mean temperature during challenge
		$P.$ is the mean pH.
1981	4	$Y_{ij} = y_{ij} - b_i(D_i - D.) - b_j(H_j - H.)$
		where Y_{ij} is the survival time corrected for the ith incubation effect and jth rearing effect
		y_{ij} is the survival time measured for the ith incubation effect and jth rearing effect
		b_{ij} is the regression coefficient for the ith incubation effect or jth rearing effect
		D_i is the ith incubation effect
		H_j is the jth rearing effect
		$D.$ is the mean incubation effect
		$H.$ is the mean rearing effect
		$X_i = x_i - b_i(D_i - D.)$
		where X_i is the length corrected for the ith incubation effect
		x_i is the length measured for the ith incubation effect
		b_i is the regression coefficient for the ith incubation effect
		D_i is the ith incubation effect
		$D.$ is the mean incubation effect
	6[a]	$Y_{a=1ij} = y_{a=1idj} - b_i(T_i - T.) - b_{jd}(H_j - H.)$
		where $Y_{a=1ij}$ is the survival time at the $a = 1$ time challenged corrected for the ith tank[b] and jth rearing effect
		$Y_{a=1ij}$ is the survival time measured at the $a = 1$ time challenged, ith day tank,[b] and jth rearing effect
		b_{i-j} is the regression coefficient for the ith tank,[b] jth rearing effect
		T_i is the ith tank[b]
		H_j is the jth rearing effect
		$T.$ is the mean tank[b]
		$H.$ is the mean rearing effect
		$Y_{a=2ijk} = y_{a=2ijk} - b_i(T_i - T.) - b_j(D_j - D.) - b_k(H_k - H.)$
		where $Y_{a=2ijk}$ is the survival time at the $a = 2$ time challenged corrected for the ith tank,[b] jth incubation effect, and kth rearing effect
		$y_{a=2ijk}$ is the survival time measured at the $a = 2$ time challenged, ith tank,[b] jth incubation effect, and kth rearing effect
		b_{i-k} is the regression coefficient for the ith tank,[b] jth incubation effect, or kth rearing effect
		T_i is the ith tank[b]
		D_j is the jth incubation effect
		H_k is the kth rearing effect
		$T.$ is the mean tank[b]
		$D.$ is the mean incubation effect
		$H.$ is the mean rearing effect

TABLE A.1. (*Concluded*)

Year	Months past post-hatch	Mathematical model
		$X_a = X_{ai} - b_a(T_a - T.)$ where X_a is the survival time corrected for the ath time challenged X_{ai} is the survival time for the ath time b_a is the regression coefficient for the ath time T_a is the ath time $T.$ is the mean time
		$U_{a=1i} = u_{a=i} - b_i(D_i - D.)$ where $U_{a=1i}$ is the length at the $a = 1$ time challenged corrected for the ith incubation effect $u_{a=1i}$ is the length at the $a = 1$ time challenged measured for the ith incubation effect b_i is the regression coefficient for the ith incubation effect D_i is the ith incubation effect $D.$ is the mean incubation effect
		$U_{a=2i} = u_{a=2i} - b_i(D_i - D.)$ where $U_{a=2i}$ is the length at the $a = 2$ time challenged corrected for the ith incubation effect $u_{a=2i}$ is the length measured at the $a = 2$ time for the ith incubation effect b_i is the regression coefficient for the ith incubation effect D_i is the ith incubation effect $D.$ is the mean incubation effect
		$Z_a = U_{ai} - b_a(T_a - T.)$ where Z_a is the length corrected for the ath time challenged U_{ai} is the length for the ath time challenged b_a is the regression coefficient for the ath time challenged T_a is the ath time challenged $T.$ is the mean time challenged
1982	5	Factorial $Y_i = y_i - b_i(T_i - T.)$ where Y_i is the survival time corrected for the ith tank[b] y_i is the survival time measured for the ith tank b_i is the regression coefficient for the ith tank T_i is the ith tank $T.$ is the mean tank
		Nested $Y_i = y_i - b_i(T_i - T.)$ where Y_i is the survival time corrected for the ith tank[b] y_i is the survival time measured for the ith tank b_i is the regression coefficient for the ith tank T_i is the ith tank $T.$ is the mean tank
		$X_i = x_i - b_i(G_i - G.)$ where X_i is the length corrected for the ith group x_i is the length measured for the ith group b_i is the regression coefficient for the ith group G_i is the ith group $G.$ is the mean group

[a]There was a source of unidentified variability present that could not be corrected with the data on hand. The likely cause was identified in January 1985 (see Discussion), and as such was an unclassified source in this data set. It should not have affected length estimates at this time, but probably will affect survival time estimates. Therefore, no h^2 for survival time was calculated.

[b]Refers to the tank to which the families were randomized for challenge.

Locomotor Activity and Blood Plasma Parameters of Acid-Exposed Lake Whitefish, *Coregonus clupeaformis*

E. Scherer, S. E. Harrison, and S. B. Brown

Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, Man. R3T 2N6

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In laboratory experiments, lake whitefish (*Coregonus clupeaformis*) were exposed to acidified water of pH 5.0, 4.7, 4.4, and 4.1 for 2 wk; controls were held at pH 7.6. Continuous recordings of locomotor activity over 4 days and nights at the end of each treatment showed a consistent trend towards reduced daytime activity and loss of diel rhythmicity with increasing ambient acidity. Feeding was observed to decrease at pH 4.7, and to cease at pH 4.4 and 4.1. Associated with these behavioral effects were changes of blood plasma variables. Plasma cortisol and glucose levels increased with lowered pH; sodium, chloride, and their fractional contribution to osmolality decreased.

Des grands corégones (*Coregonus clupeaformis*) ont été exposés en laboratoire pendant 2 sem à une eau acidifiée de pH 5,0, 4,7, 4,4 et 4,1. L'eau utilisée pour les témoins était de pH 7,6. L'enregistrement continu de l'activité locomotrice pendant les 4 jours et 4 nuits suivant chaque traitement a montré l'existence d'une tendance constante à la réduction de l'activité pendant le jour et à la perte du rythme nycthéméral en fonction de l'augmentation de l'acidité ambiante. L'alimentation diminuait au pH 4,7 et cessait aux pH 4,4 et 4,1. Ces effets sur le comportement étaient accompagnés de modifications des paramètres du plasma sanguin. La baisse du pH se traduisait par une augmentation des teneurs plasmatiques en cortisol et en glucose et il y avait déclin des concentrations de sodium et de chlorure de même que de leur contribution partielle à la pression osmotique.

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Changes in locomotor activity have often been suggested and used to detect sublethal effects of various stressors and contaminants (e.g. Fry 1947; Dandy 1970; Waller and Cairns 1972; Bengtsson 1974; Weis and Weis 1974; Percy and Mullin 1977; Scherer 1977; Scherer et al. 1979; Anonymous 1984). A number of ancillary, short-term, or qualitative observations on activity responses of fishes to acid stress exist (Beamish 1972; Mount 1973; Falk and Dunson 1977; Dively et al. 1977; Daye and Garside 1979; Ellgaard and Gilmore 1984; Lemly and Smith 1985; Jones et al. 1985a; Neville 1985), but only one paper so far (MacFarlane and Livingston 1983) focuses on quantitative activity effects, in experiments with the gulf killifish (*Fundulus grandis*).

The lake whitefish *Coregonus clupeaformis* is widely distributed over northern North America, from the northeastern United States over most of Canada and Alaska. In Canada, until very recently, this species was considered to be "... still the most valuable commercial freshwater fish... although yields have been considerably reduced in recent years because of environmental deterioration and depletion of stocks" (Scott and Crossman 1973). Little information exists on responses of this salmonid to acidification of surface waters (cf. Fromm 1980; Spry et al. 1981; Haines 1981). Harvey (1982) stated that there "is still a very modest literature on the effects of lake and stream acidification on fish behaviour, food supply and feeding, fish activity...." There are no such data concerning lake whitefish.

Numerous workers have analyzed biochemical and physiological responses of various species (including salmonids) related to acid–base balance and ionic–osmotic regulation (see reviews by Fromm 1980; Leivestad 1982). Donaldson (1981) and Schreck (1981) drew attention to the role of the hypothalamo–pituitary–interrenal and adrenergic systems in response of fish to stress. Lee et al. (1983) and Brown et al. (1984) investigated serum and plasma cortisol and glucose levels in acid-exposed rainbow trout (*Salmo gairdneri*).

Reported biochemical changes usually leave open the question as to their significance and relation to responses at higher levels of biological organization, e.g. at the integrative behavioral level (Scherer 1977; Passino 1984). In this paper we examine changes in level and diel pattern of spontaneous locomotor activity of lake whitefish exposed to acidified waters and concomitant changes of blood plasma parameters.

Materials and Methods

Experimental Fish, Water

Whitefish fingerlings of a wild stock obtained from the Dauphin River provincial hatchery near Gypsumville, Manitoba, were reared at the Freshwater Institute on commercial pellets (Martin Mills trout feed), with excellent growth and health records. At the time of testing the fish ($n = 90$) were 32.3 ± 2.0 cm long and weighed 464.2 ± 116.6 g ($\bar{x}$ ± SD).

TABLE 1. Effects of pH on locomotor activity and biochemical parameters in lake whitefish (*C. clupeaformis*) exposed for 14 d. Values represent means and standard errors of 16 fish; M = mol·L^{-1}. Asterisks denote values significantly different from controls ($P < 0.05$; Tukey's studentized range test). Also given are significance probabilities for linear and quadratic trends. NS = not significant at the 0.05 level.

pH	Activity		Cortisol (nM)	Glucose (mM)	Ca^{2+} (mM)	K^+ (mM)	Na^+ (mM)	Cl^- (mM)	Osmolality (mosmol·L^{-1})	Na + Cl/ osmolality
	Day	Night								
Control	112.7 (3.0)	57.7 (1.4)	8.3 (0.9)	3.36 (0.15)	4.17 (0.43)	3.02 (0.08)	154.7 (1.1)	131.4 (1.4)	303.3 (1.2)	94.3 (0.6)
5.0	98.9 (2.3)	52.4 (1.6)	14.3 (2.7)	3.65 (0.17)	2.44* (0.11)	2.91 (0.13)	152.5 (0.8)	125.7 (0.8)	303.8 (0.8)	91.6 (0.5)
4.7	90.7[a] (4.4)	59.2 (2.0)	45.8 (30.3)	3.87 (0.18)	2.64* (0.15)	3.80*[b] (0.10)	148.6* (1.3)	126.6 (1.2)	304.9 (1.9)	90.3* (0.7)
4.4	67.8* (3.2)	47.1 (1.5)	48.5* (15.6)	6.15* (1.07)	2.03* (0.05)	3.29 (0.14)	145.2* (2.0)	125.3* (1.7)	304.0 (2.7)	89.0* (0.8)
4.1	46.3* (2.0)	48.6 (2.1)	95.1* (46.9)	11.24* (1.50)	2.22* (0.08)	2.64* (0.14)	139.3* (2.8)	119.3* (2.1)	292.4* (2.2)	88.4* (1.0)
Linear trend	0.0157	NS	0.0001	0.0001	0.0001	NS	0.0001	0.0001	0.0005	0.0001
Quadratic trend	NS	NS	NS	0.0114	0.0086	0.0009	NS	NS	0.0005	NS

[a] n = 15; one fish died.
[b] n = 14; one hemolyzed blood sample was removed.

For holding and testing we used filtered Winnipeg tap water, dechlorinated with activated charcoal and subsequent UV radiation. This water contained 0.09 mM Na^+ (M = mol·L^{-1}), 0.03 mM K^+, 0.60 mM Ca^{2+}, 0.23 mM Mg^{2+}, 0.16 mM Cl^-, 1.39 mM dissolved inorganic carbon, and 0.03 mM free CO_2, hardness equivalent to 0.82 mM $CaCO_3$ (82.5 mg·L^{-1}). The average pH of the holding water was 7.6 (95% confidence interval 7.57–7.63). It was acidified with sulfuric acid to pH 3.9–4.0, controlled by a Radiometer TTT-2 titrator and a Bach-Simpson automatic switch. Vigorous aeration in 3000-L fiberglass tanks kept free CO_2 at <40 μM. The pH of this acidified water was adjusted upwards to the various test levels by adding 0.5 M NaOH, again controlled by a Radiometer TTT-2 titrator. This procedure raised the Na^+ levels slightly, not exceeding 0.16 ± 0.04 mM Na^+ ($\bar{x}$ ± SD) in the case of pH 5 water.

Experimental Schedule, Activity Recording

Our experimental design was a randomized block. Each treatment was tested once within each of four blocks of time, to account for possible changes in the fish's physiology over the course of the experiment. The treatment pH levels were 4.1, 4.4, 4.7, 5.0, and 7.6 (control). Groups of four fish were preexposed to the treatment pH levels in 160-L fiberglass tanks (90% water replacement in 7–7.5 h). Temperature was 14.4 ± 0.68°C ($\bar{x}$ ± SD), dissolved oxygen was always >90% saturation, light intensity (fluorescent bulbs) was 150 lx, and light periodicity was 12 h dark : 12 h light. Fish were fed 10 g of trout food pellets daily at 11:00. After exposure in these tanks for 7 d, the groups of fish were transferred to monitoring aquaria (40 × 45 × 90 cm) where the same pH, temperature, water replacement, and feeding regimes as before were continued for another 7 d. Photoperiod in these monitoring aquaria was the same as before but included 0.5-h dawn and dusk phases provided by 15-W rheostat-controlled incandescent lamps, and these aquaria were equipped with ultrasonic activity recording devices described in Scherer et al. (1979) and Scherer and van der Veen (1982). Briefly, two sound beams operating at 200 kHz (inaudible to fish, Popper and Fay 1973) were installed in each test tank horizontally, at heights of one-third and two-thirds of the 42-cm water column. Measures of fish activity were obtained by automatic recordings of the frequency of ultrasonic beam interruptions brought about by moving fish. The aquaria had opaque walls, and were placed in an enclosure to avoid visual stimulation by laboratory personnel moving about. We used one Digitec model 6140 digital recorder programmed to monitor alternate tanks for 10 min each. Thus, we obtained 30 min of recording per hour for each tank. Activity data for only the last 4 d of monitoring were used in statistical analyses to allow for recovery of fish from handling and transfer into the monitoring aquaria.

Biochemistry

Blood sampling and analysis of cortisol, glucose, and protein were similar to previously described procedures (Brown et al. 1984). To avoid netting and sampling effects, whitefish were anaesthetized directly in the test tanks with tricaine methanesulphonate (TMS, 0.2 g·L^{-1}), neutralized to tank pH with NH_4OH. Immobilization was complete within 30 s. Blood was removed from the caudal vessels within 1–2 min; plasma was immediately separated by centrifugation and stored at −20°C in polyethylene vials. Sampling was done at the end of each 14-d test or control period, at the same time of day (09:00–10:00, no feeding). Plasma osmolality was measured on duplicate 50-μL aliquots using a Precision Systems model 5004 μOsmette®. Sodium and potassium were measured with an Instrumentation Laboratory model 943 flame photometer, and plasma chloride was analyzed using a Corning model 925 chloride analyzer. Interassay coefficient of variation from duplicate analyses in at least 10 assays for osmolality, sodium, potassium, and chloride was 1.5, 3.8, 4.2, and 4.0%, respectively. Plasma calcium concentrations were estimated using a Sigma® test kit No. 586A. The validity of the Sigma kit for use with fish plasma was confirmed by atomic absorption analyses.

TABLE 2. Correlation matrix: pH, daytime activity, and blood plasma parameters, with significance probabilities beneath correlation coefficients.

	pH	Daytime activity	Cortisol	Glucose	Calcium	Potassium	Sodium	Chloride	Osmolality
Daytime activity	0.460 0.041								
Cortisol	−0.343 0.138	−0.372 0.106							
Glucose	−0.466 0.038	−0.650 0.002	0.174 0.460						
Calcium	0.791 0.0001	0.406 0.075	−0.278 0.235	−0.265 0.259					
Potassium	−0.043 0.856	0.239 0.310	−0.500 0.025	−0.307 0.188	−0.005 0.984				
Sodium	0.611 0.004	0.652 0.002	−0.103 0.665	−0.880 0.0001	0.401 0.079	−0.012 0.962			
Chloride	0.630 0.003	0.551 0.012	−0.226 0.339	−0.809 0.0001	0.560 0.010	0.166 0.483	0.750 0.0001		
Osmolality	0.228 0.332	0.630 0.003	−0.139 0.560	−0.817 0.0001	0.120 0.614	0.283 0.226	0.728 0.0003	0.669 0.001	
Na + Cl/ osmolality	0.772 0.0001	0.435 0.055	−0.125 0.598	−0.657 0.002	0.625 0.003	−0.129 0.587	0.795 0.0001	0.785 0.0001	0.279 0.234

Also, interrenal histology and measurement of nuclear diameters was performed, as described in Brown et al. (1984).

Data Analysis

Activity data were found to be normally distributed (Shapiro and Wilk 1965); variances between treatments were homogeneous according to Hartley's and Bartlett's tests (Neter and Wasserman 1974; Snedecor and Cochran 1980). Analysis of variance was then performed using the GLM procedure of the Statistical Analysis System (SAS Institute Inc. 1982) and a method appropriate to the repeated measures design (Freund and Littell 1981). Orthogonal polynomial coefficients were used to partition the treatment sum of squares into linear and quadratic components. Bartlett's test for homogeneity was also applied to the plasma data, and in some cases data were transformed according to Taylor's power law (Southwood 1978) to obtain uniform variance. Differences in plasma variables between pH levels were tested by ANOVA, and where appropriate, Tukey's studentized range test was used to distinguish differences between group means. Treatment effects were also partitioned into linear and quadratic components. For clarity of presentation, arithmetic means and standard errors have been used in Table 1. Pearson product−moment correlations were used to measure the extent of linear relationship between the locomotor activity data and the biochemistry variables (Table 2).

Results and Discussion

Levels and patterns of control activity (Fig. 1a) were very similar to recordings we had obtained before from different batches of unstressed *C. clupeaformis* in different tanks, but under similar conditions of light periodicity, light intensity, and temperature (Scherer et al. 1979). Also, in field trials with anchored, floating open-mesh cages, lake whitefish were clearly day-active, with light acting as major synchronizing factor (Scherer and van der Veen 1982). It is well known that animal activity under no-stress conditions is a rhythmic phenomenon reflecting adaptations to day/night oscillations of environmental factors, with light often acting as the major synchronizing signal or "zeitgeber" (Halberg 1960, 1975; Pittendrigh 1960; for reviews of fish data, see Schwassmann 1971; Thorpe 1978; Spieler and Kendall 1984). Consequently, normal rhythmic activity fluctuations should be taken into consideration when stress effects on activity are to be determined. A lack of suitable quantitative methods for continuous day and night recordings of activity levels and patterns of aquatic animals has probably contributed to the paucity of data on fish activity effects.

Over the range of pH levels tested, we found an easily discernable (Fig. 1a−1e) and statistically significant ($P = 0.0157$) linear trend of treatment means towards decreasing daytime activity with increasing acidity. No significant changes (linear or quadratic trends) occurred in night activity, i.e. total 24-h activity and diel rhythmicity diminished with decreasing pH. Variances of daytime activity began to increase considerably at pH 4.7.

Of the papers so far reporting quantitative activity changes caused by acidified water, Ellgaard and Gilmore (1984) give no information on day/night patterns. Experiments on *Lepomis macrochirus* were conducted "twice a day," and the data were pooled, resulting in overall rate constants for pretreatment and treatment periods (10 d in each case). Following this procedure, Ellgaard and Gilmore found a reduction of activity to approximately 25% in water of pH 4.0, compared with the activity in pH 7.5 water. Lemly and Smith (1985) monitored locomotor activity of *Pimephales promelas* over periods of 6 min each before and after presentation of a food stimulus; activity was diminished after 72 h of exposure to pH 6.5 and

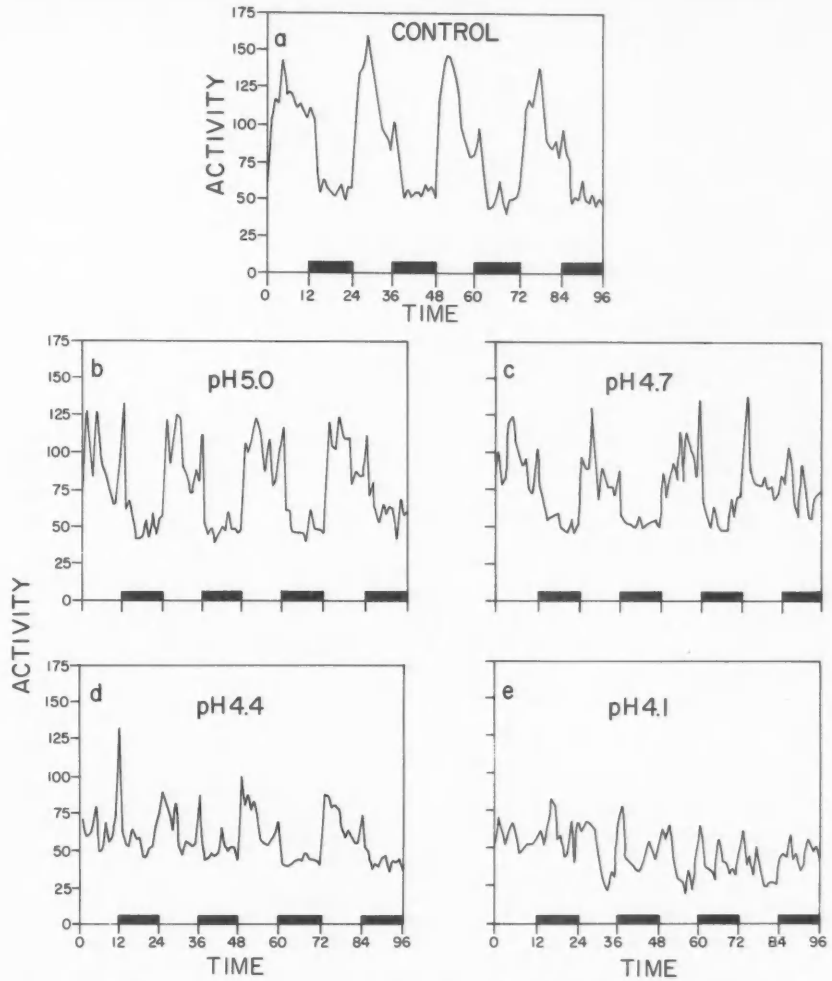

FIG. 1. Effect of acidified water on locomotor activity of *C. clupeaformis*. Activity data (number of beam interruptions per hour) are means of four replicates with four fish each. Time axis denotes number of consecutive hours monitored; black bars indicate night periods (19:00–07:00).

6.0. Jones et al. (1985a), in a somewhat similar study on *Salvelinus alpinus*, found decreases of activity at pH 4.5 and 4.75, but an increase at pH 5.0, as monitored over periods of 8 min each after 2 wk of exposure. In their study on the gulf killifish *F. grandis*, MacFarlane and Livingston (1983) used continuous day and night recordings (infrared light beam technique); they found that "rhythmicity of activity patterns was progressively reduced with increased acidification," similar to our results on *C. clupeaformis*. However, *F. grandis* was predominantly night-active before treatment, essentially maintained this pattern at pH 6.5, started to increase its activity during the normally less active day phase, and showed overall elevated activity at pH 4.0. Neither MacFarlane and Livingstone (1983) nor Ellgaard and Gilmore (1984) and Lemly and Smith (1985) controlled the liberation of free CO_2 in their acidified test waters; i.e. the possibility of pH/CO_2 interactions complicates the interpretation of these results (e.g. Höglund 1961; Höglund and Härdig 1969; Konishi et al. 1969; Scherer 1971; Neville 1979; Yoshii et al. 1980; Jones et al. 1985b).

Among ancillary and qualitative accounts, Falk and Dunson (1977) observed an initial hyperactivity in acid-exposed brook trout (lasting about 0.5 h), followed by prolonged quiescence (reminiscent of earlier observations, e.g. Höglund and Härdig 1969; Beamish 1972). Menendez (1976) found reduced activity of brook trout embryos at hatch, and Daye and Garside (1979) mentioned that posthatch locomotor activity of *Salmo salar* was progressively reduced with continued exposure to all test levels (pH 5.0, 4.5, 4.2, 4.0, and 3.7).

It may appear plausible to explain hypoactivity as caused by reduced oxygen uptake (impairment of gill function) and/or reduced oxygen carrying capacity of haemoglobin (acidosis and/or hypercapnia) resulting in reduced scope for activity; conversely, reduced locomotor activity may be interpreted as a compensatory response to reduce metabolic needs (cf. Höglund and Härdig 1969; Ellgaard and Gilmore 1984; Neville 1985).

Our plasma glucose data (Table 1) and their correlation with daytime activity (Table 2) suggest an additional hypothesis. The higher daytime activity of sight-feeding whitefish is associated with food-searching (foraging) behavior. Although the relative significance of blood glucose levels within the complex system of fish appetite regulation is not clearly understood (Fletcher 1984), the glucostatic theory (well-accepted for mammals) would suggest that elevation of plasma glucose caused by exposure to low-pH water can be expected to lead to decrease of food-search related locomotor activity, i.e. "environmental scanning" and feeding. We observed, but did not quantify, a greatly reduced food uptake at pH 4.7, while at pH 4.4 and 4.1 no feeding was observed (see also Beamish 1972; Dively et al. 1977; MacFarlane and Livingston 1983; Brown et al. 1984; Lemly and Smith 1985; Jones et al. 1985a). Essentially, this would suggest that acid-stressed whitefish are drawing on internal energy reserves, with negative implications for long-term survival. Also, reduced environmental scanning reduces the chances for selecting favorable subsets of abiotic conditions within the area inhabited.

Of the eight blood parameters analyzed (Tables 1, 2), plasma cortisol and glucose levels changed most markedly (in terms of response amplitude) with pH. Partitioning of the treatment effects showed a highly significant ($P = 0.0001$) linear trend toward increased cortisol and glucose levels with decreasing pH. The significant quadratic trend for glucose indicates that the dose–response relationship is curvilinear, due to the sudden increase in glucose at the two lowest pH levels. While Lee et al. (1983) found serum glucose increases but no cortisol changes in acid-exposed rainbow trout, dose-dependent increases of plasma cortisol and glucose were also ascertained by Brown et al. (1984). Plasma glucose increases were also documented by Nieminen et al. (1982) in pH-stressed *Coregonus peled* and *Salmo trutta*. This response may be part of a homeostatic mechanism to help maintain plasma osmolality when blood ion levels decline (McDonald 1983), or to provide a source for increased energy demand during acid stress. In conjunction with plasma cortisol and glucose increases, Brown et al. (1984) reported interrenal hyperplasia, increased nuclear diameters, and increased mitotic activity in rainbow trout at pH < 4.7 (and mortalities reaching 50% at pH 4.2). Applying the same histological examinations to our whitefish, no interrenal effects were observed (R. E. Evans, Freshwater Institute, Winnipeg, pers. comm.).

Our data indicate (see also Leivestad et al. 1976; Giles et al. 1984) highly significant ($P = 0.0001$) linear dose–response relationships for Na^+, Cl^-, and for the fraction of osmolality attributable to Na^+ and Cl^- if complete dissociation occurred ("Na + Cl/osmolality" in Tables 1 and 2). Similar to findings by Giles et al. (1984), this parameter decreased more consistently than total plasma osmolality, possibly related to an above-mentioned compensatory mechanism involving glucose.

Calcium control values were unusually high, apparently due to ripening females. If five females were removed from the sample, Ca^{2+} would drop to 3.07 ± 0.15; the pH 4.7 group would no longer be significantly different from the controls ($P > 0.05$) and there would only be a highly significant ($P = 0.0001$) linear trend with pH. Impairment of calcium regulation has been implicated in reproductive failure of acid-exposed fish (Beamish et al. 1975).

Literature data on plasma potassium responses are sparse and show, as in our study, no clear dose–response relationships (Spry et al. 1981). McDonald and Wood (1981) found in rainbow trout a small but significant increase of K^+ at pH 4.2; potassium values were lowest at pH 4.1 in our results.

Our data show a pronounced increase in response variability of day activity and cortisol at pH 4.7, and of glucose at pH 4.4, pointing to a response threshold area, with sensitivity differences between individuals.

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Role of Hypoxia in Limiting Diel Spring and Summer Distribution of Juvenile Yellow Perch (*Perca flavescens*) in a Prairie Marsh[1]

Iain M. Suthers[2] and John H. Gee

Department of Zoology, University of Manitoba, Winnipeg, Man. R3T 2N2

Suthers, I. M., and J. H. Gee. 1986. Role of hypoxia in limiting diel spring and summer distribution of juvenile yellow perch (*Perca flavescens*) in a prairie marsh. Can. J. Fish. Aquat. Sci. 43: 1562–1570.

Comparative field and laboratory data on the distribution-limiting levels of dissolved oxygen (DO) for yearling yellow perch (*Perca flavescens*) is presented. At Blind Channel, Delta Marsh, Manitoba, diel fluctuations in DO concentrations began by June, approximately 6 wk after ice-off. By early July severe hypoxia (≤1.5 ppm DO) first occurred in the cattail habitat close to the substrate at dawn, and by early August severe hypoxia extended throughout the cattail bed. Using wire minnow traps, juvenile perch persisted within the cattails close to the substrate in June. In early July, juvenile perch occupied the submerged macrophyte – open water habitat, away from the cattail and hypoxia. Significant diel changes in horizontal distribution were evident at one site, where fish avoided severe hypoxia in the cattail bed overnight but returned during the day, as there was little alternative cover and when DO levels were not lethal. In situ survival experiments demonstrated decreased survival close to the substrate, in the cattail, and overnight compared with overday, reflecting the distribution of low DO. Survival increased 27% over the control by bubbling oxygen into holding cages. In a two-chambered normoxic/hypoxic tank, perch demonstrated a preference for cover, and avoidance of hypoxia at 1.5–3.0 ppm DO. The habitat choice of juvenile yellow perch in Delta Marsh is a compromise between the cattail, with favorable predator/prey conditions, and hypoxia.

Les auteurs présentent des données comparatives, obtenues sur le terrain et en laboratoire, sur la distribution des teneurs limitantes en oxygène dissous (OD) pour des perchaudes (*Perca flavescens*) d'un an. Dans le chenal Blind de Delta Marsh (Manitoba), les fluctuations nycthémérales de la concentration en OD ont débuté en juin, environ 6 sem après la fonte des glaces. Au début de juillet, on notait pour la première fois une importante hypoxie (≤1,5 ppm OD) à l'aube dans l'habitat à typhas, à proximité du substrat; au début d'août cette hypoxie s'étendait à toute la zone à typhas. L'utilisation de trappes à ménés métalliques a permis de noter que les perchaudes juvéniles demeuraient dans les typhas, près du substrat, en juin. Au début de juillet, les poissons occupaient l'habitat d'eau libre à macrophytes submergés, loin des typhas et des conditions hypoxiques. Des variations nycthémérales significatives de la distribution horizontale ont été notées en un endroit où les poissons évitaient les conditions hypoxiques sévères des typhas au cours de la nuit, mais y retournaient durant le jour car il y avait peu de couvert disponible et les concentrations de OD n'y étaient pas mortelles. Des essais de survie *in situ* ont montré une baisse de la survie à proximité du substrat dans la zone à typhas; cette baisse était plus importante la nuit que le jour, ce qui reflète la distribution des faibles teneurs en OD. Le taux de survie a pu être accru de 27 %, comparativement à celui des témoins, en diffusant de l'oxygène dans les cages. Au cours de ces essais en cuves à deux compartiments (conditions normales et hypoxie) les perchaudes ont préféré le couvert et évité une hypoxie de l'ordre de 1,5–3,0 ppm de OD. L'habitat choisi par les perchaudes juvéniles de Delta Marsh représente un compromis entre la zone à typhas où les conditions prédateurs–proies sont favorables et l'hypoxie.

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Habitat criteria for juvenile freshwater fish include predator risk, prey abundance, presence of competitors, temperature, and other factors (e.g. Crowder et al. 1981; Hall and Werner 1977; Keast et al. 1978). Werner et al. (1983) demonstrated with centrachids a tradeoff between prey availability and predator risk in habitat choice. Habitat choice is a compromise among many factors, including the interaction of physical and biological effects. The potential for low dissolved oxygen (DO) to strongly interact with the biological factors has rarely been considered (Rudstam and Magnuson 1985), and yet hypoxia is a pervasive phenomenon in many aquatic communities. Severe hypoxia in potholes and small eutrophic lakes caused by collapse and subsequent decomposition of large algal blooms (e.g. Nicholls et al. 1980) results in catastrophic mortality of fishes in midsummer, as well as mortality due to nocturnal hypoxia and subsequent avian predation (Kushlan 1974; Tramer 1977). These accounts described a characteristic sequence of mortality, related to the species' physiological and morphological adaptations for breathing the surface film or air-breathing (Gee 1980). An obvious, but often ignored, initial behavioral response to hy-

[1]Contribution No. 136 of the University of Manitoba Field Station (Delta Marsh).

[2]Present address: Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1.

poxia is movement to habitats of higher DO (Hochachka 1980).

Seasonal changes in vertical distribution and survival of fish in relation to low DO (and high temperature) occur in stratified lakes, where by midsummer the hypolimnion may become anoxic (Clady 1976; Engel and Magnuson 1976; Vigg 1980; Rinne et al. 1981; Rudstam and Magnuson 1985). Horizontal DO gradients, resulting from sewage effluent or natural causes, also affect fish and invertebrate distribution (Odum and Caldwell 1955; Alabaster 1959; Birtwell et al. 1983). Magnuson et al. (1985) showed that vertical and horizontal fish distribution under ice was a function of low DO and high CO_2. Northern pike (*Esox lucius*) can avoid low DO in late winter (Casselman 1978), and juvenile pike are attracted to traps with an aerated discharge under the ice (Johnson and Moyle 1969). Hypoxia and fish scarcity may be correlated but the underlying mechanisms are difficult to establish in the field (Whitmore et al. 1960) because of other interacting factors (prey distribution, light levels). Rarely are these hypotheses tested with in situ survival experiments (Odum and Caldwell 1955; Klinger et al. 1982; Birtwell et al. 1983). Comparative laboratory and field studies on the effect of low DO on fish distribution are essential to resolve these interacting effects, particularly with respect to a changing diel regime. Alabaster and Robertson's (1961) study is unique in using a large outdoor tank to study diel effects of temperature, DO concentrations, and light levels on fish activity and distribution, though the results were inconclusive.

Our objectives were to describe (1) the temporal and spatial variation in DO, temperature, and vegetation and (2) the associated distribution of yearling yellow perch (*Perca flavescens*) in Delta Marsh. We demonstrate experimentally how amounts of DO and cover affect fish distribution and survival in the laboratory and in the field. Delta Marsh is one of the largest freshwater marshes in North America and surprisingly little is known of its fish populations. The marsh is a nursery ground for juvenile northern pike, carp (*Cyprinus carpio*), spottail shiner (*Notropis hudsonius*), fathead minnow (*Pimephales promelas*), and brook stickleback (*Culaea inconstans*). The marsh is potentially hypoxic due to the abundance of decaying vegetation and submerged macrophytes, but during the spring it provides fish with cover, protection from storms, and a more abundant invertebrate food resource compared with the adjacent Lake Manitoba.

Materials and Methods

Study Area

Research was conducted from the University of Manitoba Field Station, Delta Marsh (50°11′N, 98°23′W), situated on the southern shore of Lake Manitoba. Data were collected at Blind Channel, a former bed of the Assiniboine River. It is a shallow (1-m maximum depth), 8-km meandering channel lined with cattail (*Typha* spp.) and bullrush (*Scirpus* sp.). The only connection to Lake Manitoba is via a narrow cut, midway along the channel. Average water levels in Blind Channel vary by ±15 cm due to wind-generated seiches on Lake Manitoba. Water chemistry is similar to that of the lake (pH 8.0−8.5, brackish, ≥1500 mg total dissolved solids·L^{-1}) and turbid, particularly in spring but clearing by early summer (Tudorancea and Green 1975).

Two sites (A and B) were selected on the basis of accessibility and presence of large stands of cattail vegetation. Cattail was selected over stands of bullrush, as yellow perch were more abundant in the former (Suthers 1984). In June and July there was a greater percent cover of submerged macrophytes in the open water habitat at site A than at site B. Site A at the northern extremity of Blind Channel was comparable with a backwater, with little current and abundant macrophytes (*Myriophyllum* sp., *Potamogeton* spp., and *Utricularia* sp., Table 1), while site B was midway along the channel, with sparse submerged macrophytes (chiefly *P. pectinatus*) and slight current due to wind seiches.

TABLE 1. Percent cover of submerged macrophytes at station 3 (open water habitat) in 1983 during the periods of yellow perch abundance (n = 20 counts ± SD). Early June values included dead plants from the previous summer.

Period	Site A	Site B
Early June	51.5±29.4	3.3±5.7
Late June	31.6±35.8	1.8±2.2
Early July	64.3±41.0	31.8±28.1
Late July	—[a]	61.0±34.8

[a]Not recorded, approximately 100%.

Sampling Procedures

The DO regime and fish distribution were determined at sites A and B in 1982 along four replicate transects selected at random from a total of 10 set perpendicular to the shore, approximately 25 m apart. Each transect was composed of four stations. Station 1 was approximately 2 m into the cattail bed in 30−40 cm of water, station 2 was at the edge of the cattail in 40−50 cm of water, station 3 was 2−3 m offshore in 50−60 cm of water, and station 4 was almost midchannel in 70−80 cm of water. At each station a rod supported two wire minnow traps (42 cm long; 21 cm diameter) with one at the water surface and one at the substrate. For 3 d four transects were selected and traps set in the morning (07:00−10:00 and then examined that evening (19:00−22:00) when a new set of four transects was selected. These times were intended to approximate midpoints of the diel DO fluctuation, thus generally trapping over a high DO and a low DO time of day. This procedure was repeated the following week at site B. The 3-d sampling intervals at each site were repeated over four sampling periods during the summer: 8−18 June, 6−16 July, 27 July − 6 August, and 17−26 August.

For each trap, DO and temperature were recorded and fish were counted before release 500 m away. DO was recorded using a YSI oxygen meter (model 57) and calibration was checked after each transect. Comparison with the Winkler method indicated close agreement (±4%). There were five levels of treatment for each site: sampling period (4), day (3), time of day (2), station (4), and depth (2) with four replicate transects. DO was analysed for each site with a five-way factorial analysis of variance (ANOVA). Nearly all factors and interactions were significant and only the six largest sums of squares are discussed.

The catch per trap was expressed as a proportion of each morning's or each evening's total catch. This removed the effect of larger catches overday than overnight (Suthers 1984) and greater abundance in early June than early July. Homogeneity of variance was attained using an arcsine, square-root transform of this proportion. The distribution of yellow perch

was analysed for each site using the larger, bottom trap catches during early June and early July sampling periods in a four-way ANOVA (period (2), day (3), time of day (2), by station (4)).

In 1983, five transects were maintained at each site with three stations per transect (omitting station 4 in midchannel). DO, temperature, and catch were recorded at each trap at 04:00, 10:00, 16:00, and 22:00 for 2 d at each site during six sampling periods during the summer: 16–26 May, 30 May – 7 June, 11–17 June, 26 June – 6 July, 19–27 July, and 15–19 August. DO was analysed for each site in a five-way factorial ANOVA (period (6), day (2), time of day (4), station (3), depth (2) with five replicate transects). A continuous 24-h record of temperature and DO (YSI model 56) was obtained at stations 1–3 at site A during 1983.

The abundance of yearling yellow perch was lower in 1983 so the 04:00 and 10:00 catch and the 16:00 and 22:00 catch were combined to give an "overnight" and an "overday" catch, comparable with 1982. Catches were converted to proportions as above and an identical analysis was applied to the larger bottom trap catch, omitting the May and August sampling periods (period (4), day (2), time of day (2), station (3), and transect (5)).

Experimental Manipulations in the Field

Few fish were trapped amongst the cattail by early July, and experimental manipulations were used to determine if fish could survive in the cattail habitat at that time. Caged fish were held at site A in mid-July 1983 for two days and two nights along two replicate transects composed of stations 1, 2, and 3, top and bottom. Each cage (a minnow trap, corked at both ends) contained five perch with the top cage just below the water surface. Fish were caught from Blind Channel in trap nets within 24 h of the experiment. At approximately 10:00, DO, temperature, and number of live fish were recorded, the traps stocked with new fish, and reexamined at 22:00.

To determine if the level of DO affected survival in the cattail, the oxygen regime was manipulated over two days and two nights in early July 1983 under three treatments: oxygenated (O_2 bubbled), hypoxic (N_2 bubbled), and control. The experimental design was identical to the experiment described above except that station 3 was omitted. Oxygen and nitrogen were supplied from cylinders lying in a nearby dinghy to airstones in the bottom trap. To reduce large-scale mixing with surrounding water, a small plastic sheet was placed under the bottom trap and over the surface trap. After each 12-h period, DO and number of live fish were recorded. Analysis of the percent survival (arcsine transformation) involved a four-way factorial ANOVA (time of day (2), treatment (3), station (2), depth (2)).

Analyses of variance were performed using the Statistical Analysis System (SAS 1982) and differences in the F statistic were considered significant at the 5% level ($p < 0.05$). Taylor's power law (Elliott 1977) was applied to determine a suitable transformation and checked using Cochran's test. Comparison of means was performed using Duncan's multiple range test.

Laboratory Experiments

Yearling yellow perch (6–10 cm fork length) were caught in Blind Channel in July 1982 and maintained at 12°C in a 12 h light: 12 h dark photoperiod on frozen brine shrimp (maintenance ration) until January 1984, when the fish were acclimated to 18°C over 4 wk to approximate June water temperatures in the marsh.

The test chamber was a 120 × 26 × 15 cm clear acrylic plastic tank with an intake at each end, draining at the centre from holes in the sides and bottom (Scherer and Nowak 1973). Deoxygenated water (generated by bubbling nitrogen through a 200-L drum) flowed to one side of the test-chamber while the opposite side remained normoxic. Flow rates of about 0.8 $cm \cdot s^{-1}$ (approximately 10 $L \cdot min^{-1}$ at each end) yielded distinct conditions of DO in the two halves of the tank through which fish swam without impediment. To simulate conditions in the marsh, the hypoxic side contained cover (weighted black plastic strips), the normoxic side being bare. DO was monitored (YSI model 57) with the probe next to the hypoxic side inlet. A clear plastic sheet covered the surface to prevent atmospheric exchange. The tank was illuminated by a row of incandescent bulbs with a diffusing screen (35 klx at the surface) under a 12 h light: 12 h dark photoperiod, and also illuminated continuously by four infrared lamps (400 nm). Observations were made on a school of eight fish, acclimated to the test tank for 3 d. Using a Sony video camera with a Vidicon sensor tube, and a Sony SL 5400 VCR, the number of fish on the hypoxic side (with cover) were counted every 30 s for 15 $min \cdot h^{-1}$ over two control and two experimental nights (alternated, using the same school). The experimental nights involved lowering DO 1.5 $ppm \cdot h^{-1}$, starting at midnight, from saturation (9.2 ppm) to 0.6 ppm at 06:00. After the incandescent lights came on the count was repeated at 07:00 and DO was returned to saturation by 13:00 at the same rate.

Results

Dissolved Oxygen and Temperature Regimes

DO concentrations varied considerably in time and space within the study area. Using DO (1982) as the dependent variable, the six largest sums of squares explained almost 75% of the total variance (Table 2). Distance from the cattail ("station") was the most important single factor at sites A and B, the lowest DO being in the cattail and increasing offshore to station 4. DO recorded in the evening was greater than the morning ("time of day"), and also greater at the surface than at the substrate ("depth"). Generally, mean DO was higher and more variable at site A than at site B (Table 2, total SS). Daily water temperature changed ±2° about the mean but increased seasonally from 9.5°C in late May to 22°C by August. Extreme conditions were recorded in late summer, with temperatures on occasion as high as 27°C and 18 ppm DO (200% saturation). The 1983 DO analysis (Table 2) for sites A and B also showed greater DO offshore, but "period" accounted for a larger proportion of the variance, as six periods were sampled during the 1983 summer (which was comparatively warm and dry). DO was highest in the midafternoon (16:00 > 22:00 > 10:00 > 04:00). Significant diel fluctuations in DO occurred from mid-June onwards (site A) and early July onwards (site B) compared with earlier, more uniform conditions.

Severe hypoxia (≤1.5 ppm DO) was first evident in early July at the station 1 bottom trap position at dawn. By early August severe hypoxia extended throughout the cattail bed for most of the day. Stations 3 and 4 never became hypoxic (1.5–3.0 ppm). These patterns in DO and temperature regimes are summarized in a series of continuous recording traces (Fig. 1) from May, June, and July 1983 at site A. In May, DO

TABLE 2. Six factors with the largest sums of squares of a five-way ANOVA with DO as the dependent variable at sites A and B during 1982 and 1983. All are significant at $p \leq 0.005$

Source	df	Site A		Site B	
		SS	% total SS	SS	% total SS
1982					
Period	3	1 235	9.5	695	8.6
Period × replicate day	6	394	3.0	104	1.3
Time of day	1	2 992	23.0	1 679	20.7
Station	3	3 618	27.8	2 221	27.3
Depth	1	1 218	9.3	903	11.1
Period × depth	3	164	1.6	301	3.7
Residual	576	2 048	15.7	1 161	14.0
Total	767	13 028	100.0	8 124	100.0
1983					
Period	5	8 842	38.7	2 788	18.6
Time of day	3	2 263	9.9	2 475	16.5
Period × time of day	15	685	3.0	671	4.5
Station	2	3 019	13.2	3 164	21.1
Depth	1	2 481	10.8	1 403	9.4
Time of day × depth	3	921	4.0	339	2.3
Residual	1151	1 579	6.9	1 952	13.0
Total	1438	22 873	100.0	14 987	100.0

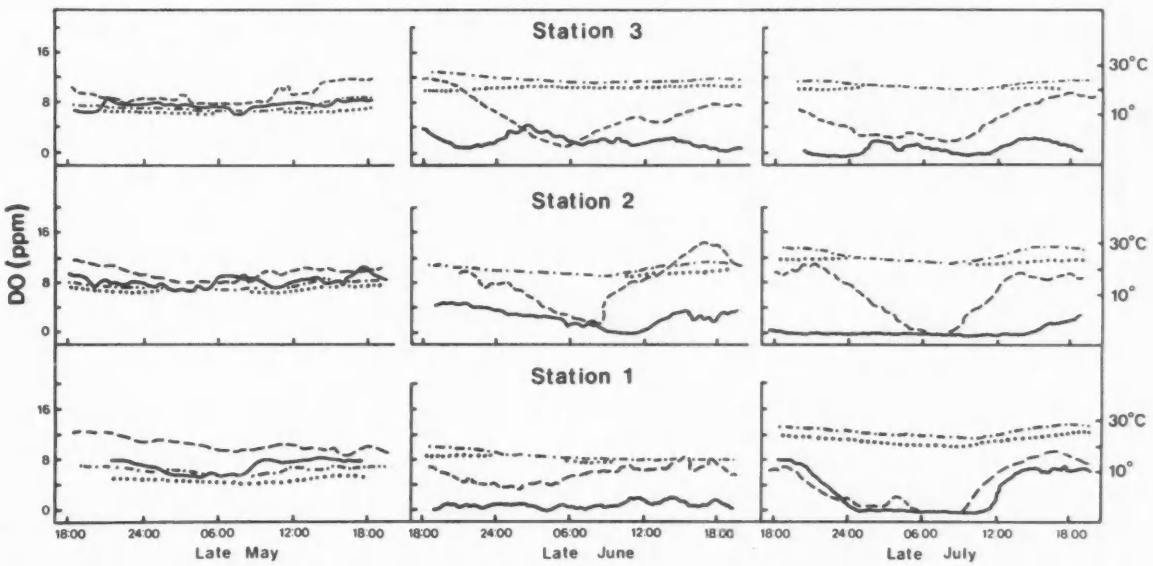

FIG. 1. DO (solid lines) and temperature (dotted lines) traces from a continuous recorder at site A, 1983, at top and bottom traps. Top and bottom temperatures are dot–dash and dotted lines, respectively; top and bottom DO values are dashed and solid lines, respectively.

conditions were uniform both spatially and temporally, but in June and July, DO increased with distance from shore and from the substrate and with increasing diel fluctuations.

Yellow Perch Distribution

Most perch were found in the bottom trap and were less abundant in 1983, as shown by a comparison of the mean catch per 12 h per trap (n = number of traps set):

Trap location	Top	Bottom
1982 (n = 768)	0.429	4.701
1983 (n = 1200)	0.020	0.342

Generally, bottom trap catches were greater amongst the cattail than open water in early June, but in early July, yellow perch began to move offshore, and by August, catches were smaller and mostly at open water stations.

The four-way ANOVA of the 1982 proportion of catch in the bottom traps showed two significant effects at site A and four at site B (Table 3). These results indicate the following. A seasonal shift in distribution was demonstrated at both sites by significantly larger catches at station 1 in early June relative to early July when there were larger catches at station 2. A daily shift in distribution was evident at site B over both periods by significantly larger catches at station 1 overday than overnight

TABLE 3. Significant factors of a four-way ANOVA with proportion of yellow perch caught in the bottom trap as the dependent variable at sites A and B during 1982 and 1983 (**$p \leq 0.005$).

Source	df	Site A SS	Site A F	Site B SS	Site B F
1982					
Station	3	1.3350	18.7**	1.4694	17.8**
Period × station	3	1.5736	22.0**	0.7942	9.6**
Day × station	6	0.1153	0.8	0.5649	3.4**
Time of day × station	3	0.0351	0.5	0.4835	5.9**
Residual	142	3.3859		3.9599	
Total	189	7.1563		8.2227	
1983					
Period × station	3	1.7428	3.73**	1.8116	5.00**
Residual	192	14.9576		11.5999	
Total	239	18.8234		15.8641	

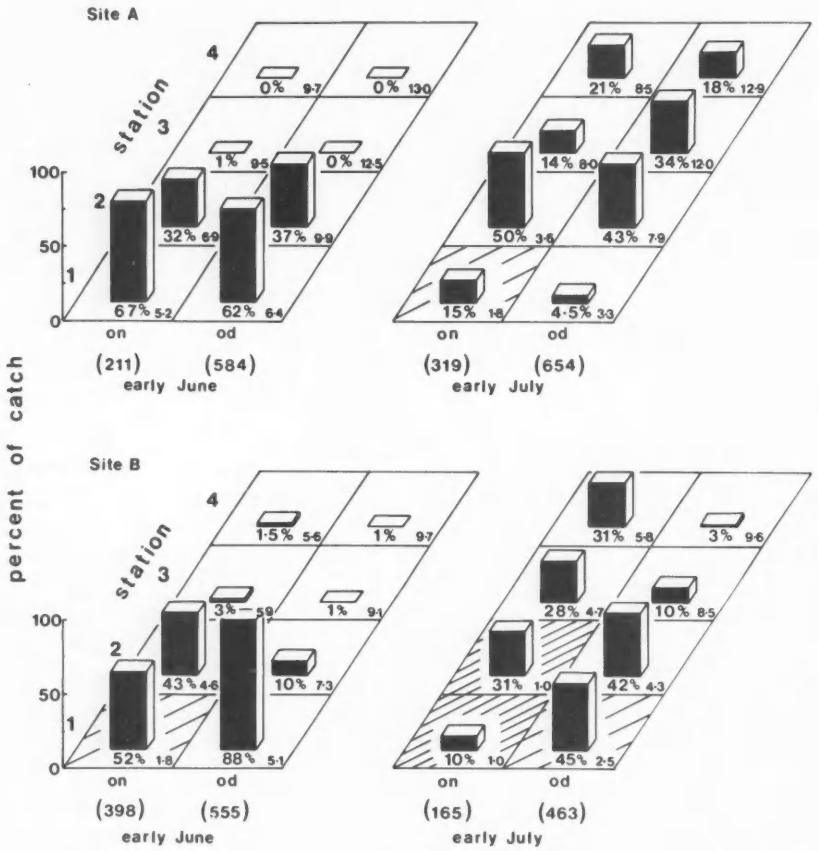

FIG. 2. Percent of each overnight and each overday bottom trap catch (n = 12) for yearling yellow perch in early June and early July 1982 at sites A and B. Total overnight and overday catch for the 3 d and four replicate transects are in parentheses. Corresponding average DO measurement is beside each column. Shaded, severely hypoxic (≤1.5 ppm); hatched, hypoxic (1.5−3.0 ppm); on, overnight catch 09:00−21:00; od, overday catch 21:00−09:00. Duncan's multiple range test ranked the period × station means for sites A and B and the time of day × station means for site B as follows. A: June 1 > July 2 = June 2 > July 3 > July 4 > July 1 > June 3 = June 4; B: June 1 > July 2 > June 2 = July 1 = July 3 = July 4 > June 4 = June 3; B: overday 1 > overnight 2 = overday 2 = overnight 1 > overnight 3 = overnight 4 = overday 3 = overday 4.

TABLE 4. Mean percent survival ($n = 4$) of five yellow perch (±SE) caged overday (10:00–22:00) and overnight (22:00–10:00) at stations 1, 2, and 3 at both depths. Mean DO after each 12-h treatment is shown. Daily O_2 regime is that of mid-July 1983. Mean water temperature is 24.2°C

	Overnight		Overday	
Station	% survival	DO (ppm)	% survival	DO (ppm)
3 surface	100	6.2	95 (±5)	9.8
substrate	80 (±20)	3.6	90 (±10)	4.8
2 surface	0	4.2	85 (±10)	9.5
substrate	0	0.8	25 (±25)	1.8
1 surface	0	0.4	0	6.7
substrate	0	0.2	5 (±5)	1.6

TABLE 5. All significant factors in a four-way ANOVA with the proportion of surviving yellow perch as the dependent variable near site A in early July 1983, overday and overnight under three treatments: control, hypoxic, and oxygenated (Fig. 3). (*$0.01 < p < 0.05$; **$p < 0.01$).

Source	df	SS	F
Time of day	1	3.45	14.01**
Treatment	2	11.48	23.32**
Time of day × treatment	2	1.78	3.61*
Station	1	1.91	7.74**
Depth	1	3.40	13.80**
Residual	66	16.25	
Total	89	41.63	

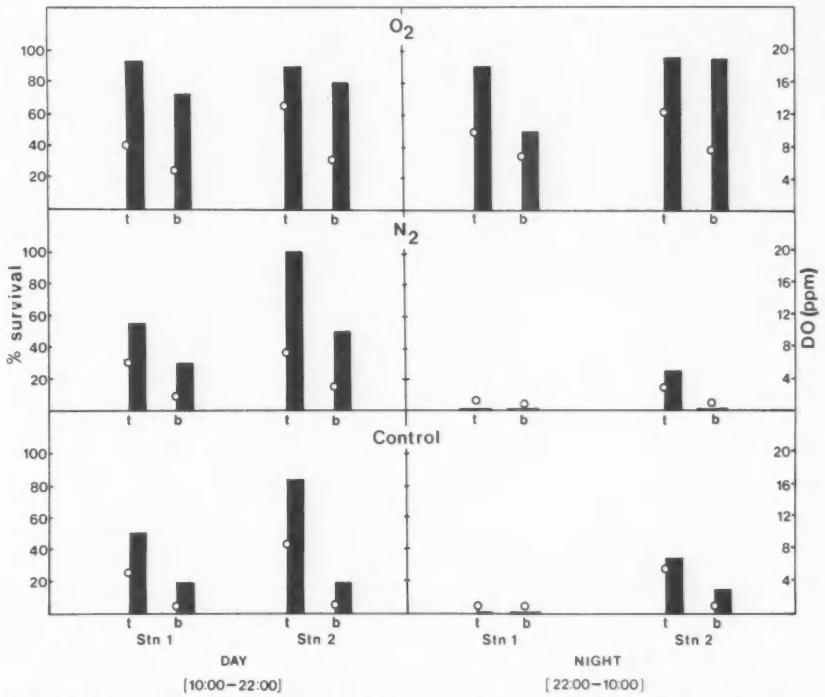

FIG. 3. Average percent survival ($n = 4$) of five yellow perch caged overday and overnight at stations 1 and 2 at both depths under three treatments: oxygen bubbled, nitrogen bubbled, and control. Average DO after each 12-h treatment is shown by an open circle. The environmental DO regime ("control") is that of early July 1983, with average water temperature of 25°C. Duncan's multiple range test ranked the treatment × time of day means as follows: O_2 day = O_2 night > N_2 day > control day > N_2 night = control night.

(Fig. 2). There were also significant day to day shifts in distribution and a slight but not significant period effect on diel distribution ($p = 0.08$) at site B (Table 3).

Analysis of the 1983 proportion data also showed a significant seasonal change in the fish distribution (Table 3), with significantly less fish caught in the cattail habitat in early July. The combined morning and evening catches were low, yet there was some indication of a changing diel distribution in July, as fish were caught at station 1 during the day, but not overnight.

The average DO corresponding to each average catch indicates that yellow perch are present where DO may increase to no more than 2.0–3.5 ppm. Perch are absent when mean DO was 1.5 ppm or less at the time of sampling.

In Situ Survival Experiments

The survival of caged fish also reflects the distribution of low DO. Yellow perch survived overnight only at the open water station (station 3) in the control transects during mid-July (Table 4). However, during the day most individuals survived at station 2 in the surface trap, fewer surviving in the bottom trap, and none surviving at station 1. Although high DO con-

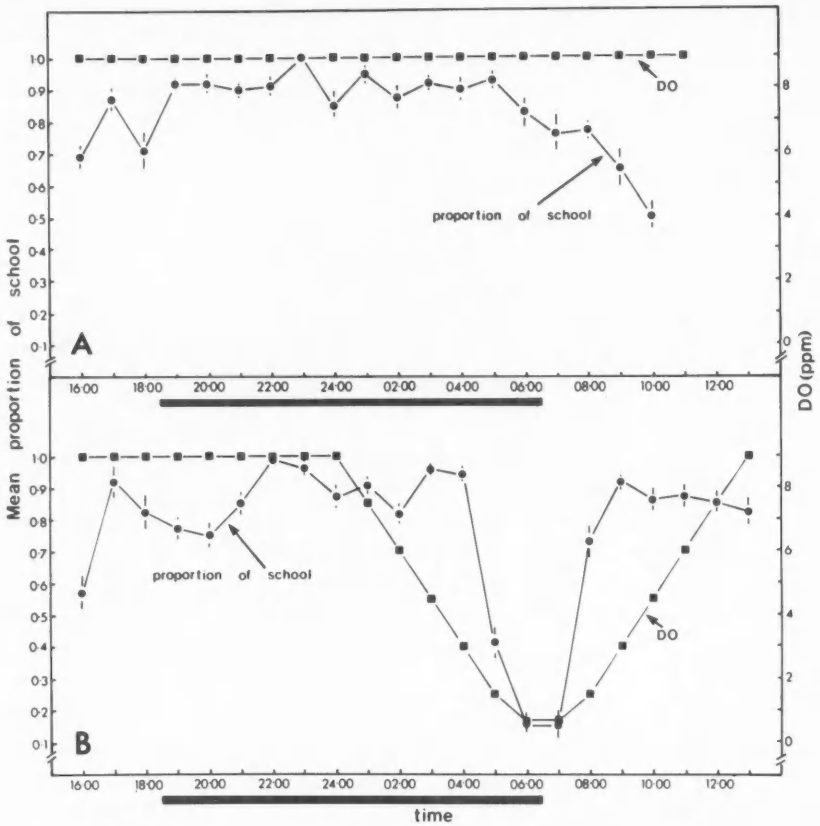

FIG. 4. Average proportion (n = 30 counts) of the school of eight fish on the hypoxic side (with cover) over two replicate runs under a day/night regime. (A) Control; (B) increasing and decreasing hypoxia; lights off at 18:15 and on at 06:15. Vertical lines indicate 95% confidence limits.

centration was recorded for the station 1 surface trap by evening (Table 4), lethal levels of DO were present in the late morning, contributing to the total mortality of yellow perch. Thus, by mid-July, stations 1 and 2 were uninhabitable overnight.

In the manipulated DO experiments the four factors and the interaction term (time of day × treatment) were significant (Table 5). There was significantly greater survival in the surface trap and at station 2 than at station 1 (Fig. 3). Survival under the oxygen-bubbled treatment during day and night was significantly greater than the nitrogen-bubbled and control transects, and in the two latter treatments, survival was significantly greater overday than overnight.

Laboratory Experiments

Yellow perch demonstrated a marked preference for cover throughout the day and night (Fig. 4A). During increasing hypoxia (Fig. 4B), fish avoided the hypoxic side with cover when DO fell to between 3.0 and 1.5 ppm and returned under increasing DO at the same concentration during daylight conditions.

Discussion

Diel fluctuations in DO concentrations develop at Delta Marsh during summer such that by late June, severe hypoxia (≤1.5 ppm) occurs at dawn amongst the cattail. Catches of yearling yellow perch indicated seasonal and diel shifts in distribution between the preferred cattail and open water habitats. It is proposed that these changes in distribution are a direct result of severe hypoxia and the growth of alternative cover in the open water habitat.

Yellow Perch Distribution

The marked seasonal movement away from cattail in midsummer and the high mortality of yellow perch caged in the cattail at this time directly parallel spatial and temporal changes in DO concentrations. This seasonal change in distribution is directly comparable with avoidance by fish, in some eutrophic lakes, of the anoxic hypolimnion in late summer (e.g. Clady 1976; Engel and Magnuson 1976; Vigg 1980). In addition, growth of submerged vegetation in the offshore open water habitat by early July offered alternative cover without the same conditions of hypoxia. Werner et al. (1983) established that cover was a major factor affecting the distribution of juvenile bluegill sunfish (*Lepomis macrochirus*), offering protection from predators, despite greater foraging success in the open water. In Blind Channel, northern pike were the predominant fish predator enhancing continuous, not diel, preference for

cover. Predatory aquatic insects were abundant in Blind Channel but their effect on yellow perch is unknown.

Juvenile yellow perch feed predominantly on zooplankton (particularly Cladocera), aquatic insect larvae, and to a lesser extent chironomids during the spring and summer (Keast and Welsh 1968; Keast 1977; Kelso and Ward 1977; Paszkowski and Tonn 1983; I. M. Suthers, pers. obs.). At both sites in 1983, activity trap estimates of zooplankton abundance in early June were two orders of magnitude greater in cattail than in open water (I. M. Suthers, unpubl. data). Thus, in Blind Channel, the cattail habitat provides cover and, at least during June and July of 1983, a more abundant food resource. Yet the suitability of this habitat is compromised as shown by the survivorship of caged fish which was due to hypoxia demonstrated by the manipulated DO regimes. Bubbling oxygen amongst caged fish greatly increased survival even under the most hypoxic conditions.

A changing diel distribution of fish in direct response to diel variations in DO has not been conclusively demonstrated in other studies, as there are often other interacting factors such as light level and temperature (Vigg 1980; Rinne et al. 1981). In this study, there was a diel change in distribution away from the cattail habitat at night and returning during the day in both early June and July at site B but not at site A in 1982. Site A, at the end of Blind Channel, had warm, still conditions, a faster decay of the dead cattail with more variable DO concentrations but with luxuriant growth of submerged macrophytes in the open water habitat. In early July, hypoxia was avoided by inhabiting this alternative cover. At site B, hypoxic conditions developed overnight during early June and early July, but the only substantial cover (particularly in early June) was amongst the cattail. Consequently, yellow perch tended to return there during the day with increased light level and predator activity and higher DO levels. This site is midway along Blind Channel, permitting slight two-way water movement by wind-generated seiches. Here, the movement of more turbid lake water possibly retarded growth of submerged macrophytes, and also swept away detritus leaving little cover in the open water habitat (Table 1). These conditions were modelled in the laboratory, which showed that while cover was the main habitat requirement, yellow perch distinctly avoided water at 3.0–1.5 ppm, in accordance with the field data. Alabaster and Robertson (1961) demonstrated in four trials in an outside tank that adult yellow perch avoided DO concentrations at 6.7, 3.0, 2.3, and 1.9 ppm (23°C). These values are all higher than incipient lethal DO levels, which for post-young-of-the-year yellow perch (10–25°C), using a variety of techniques, were below 1 ppm (Burdick et al. 1957; Doudoroff and Shumway 1970; Petit 1973; Gee et al. 1978).

Field observations of juvenile and adult yellow perch in lakes have shown crepuscular increases in activity (Craig 1977; Kelso and Ward 1977), often associated with an onshore movement in the evening and offshore in the morning (Hasler and Bardach 1949; Hasler and Villemonte 1953; Scott 1955; Emery 1973; Engel and Magnuson 1976). This is contrary to the diel movement at site B, which is argued to be in response to hypoxia. There is no evidence for a light-induced onshore/offshore migration at site A, as minnow trap catches show cover to be the major requirement during the day and night in early June, when there was no hypoxia. Direct observation of this was not possible due to low visibility in June and the dense emergent vegetation. There were no marked temperature gradients of any magnitude, and the water is hard and well buffered, reducing gradients in pH and free CO_2. Shelford and Allee (1913) and Hoglund (1961) argued that in the field, fish may detect CO_2 and thus avoid low DO concentrations.

Brook stickleback caught with yellow perch also exhibited a seasonal shift offshore, as well as a change in diel distribution in July by moving both horizontally and vertically away from severe hypoxia. Complete avoidance of the station 1 bottom trap was evident when mean DO was below 1 ppm at time of sampling, and was associated with an increased surface trap catch (Suthers 1984).

Factors Modifying Level of DO Avoidance

The precise level of DO that limits yellow perch distribution in Delta Marsh cannot be determined from field data. The DO regime preceding each 12-h sampling can only be inferred from the DO reading at each trap, and from the continuous recording (Fig. 1), which shows a general decrease from 21:00 to 06:00. The rate and exent of this decrease depends not only on habitat but would vary daily and spatially with microhabitat variation in DO level. Consequently, regressions of mean station 1 catch on mean DO concentration at sites A and B explained only 62 and 40% of the catch variance, respectively. Yellow perch may make brief feeding forays into the hypoxic cattail by exploiting microhabitats with elevated DO concentrations. Both Coulter (1967) and Dendy (1946, cited in Doudoroff and Shumway 1970) gillnetted fish in severely hypoxic benthic regions, presumably feeding. Centrachids were found with food items in their gut that could only have been obtained by foraging in the hypoxic hypolimnion (Dendy 1956; Hanson and Qadri 1984). Microhabitat variation in DO concentration could depend on patches of photosynthetic O_2 production and consumption amongst the cattail. Microzones of high DO may also occur around bubbles produced by photosynthesis that are trapped under algae and displaced patches of periphyton. Exploitation of these microzones by fishes would involve both a physiological (activity level, heart rate, ventilatory rate and amplitude) and a behavioral response to environmental cues (sunlight, vegetation, odor). For example, exploitation of high oxygen microzones at the ice–water interface due to cracks and bubbles was observed in mudminnows and brook stickleback under simulated ice conditions (Klinger et al. 1982; Magnuson et al. 1982). In a naturally anoxic hot-spring, Odum and Caldwell (1955) observed fish to exploit eddies where DO had increased slightly.

The hypothesis that changes in distribution of yellow perch are a direct result of severe hypoxia and the growth of alternative cover in the open water habitat is supported by simulation of these events in the laboratory, the in situ survival experiments, and consideration of crepuscular behavior and other factors. The diel shift in distribution is not a periodic, predictable migration but an avoidance of low DO concentrations that can occur between spring normoxic conditions and midsummer anoxia. The optimality tradeoff between prey and predator distribution (Werner et al. 1983) now appears as one between favorable predator/prey conditions and hypoxia. Assessment of juvenile fish utilization of the productive littoral zone as nursery areas, or as refugia in predation studies, should be made in the context of a temporally varying environment.

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Trophic Relationships in Freshwater Pelagic Ecosystems

Donald J. McQueen and John R. Post

Department of Biology, York University, 4700 Keele Street, Toronto, Ont. M3J 1P3

and Edward L. Mills

Department of Natural Resources, Cornell University, Ithaca, NY 14853, USA

McQueen, D. J., J. R. Post, and E. L. Mills. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. 43: 1571–1581.

Relative impacts of bottom-up (producer controlled) and top-down (consumer controlled) forces on the biomass and size structure of five major components of freshwater pelagic systems (piscivores, planktivores, zooplankton, phytoplankton, and total phosphorus availability) were estimated. Predictions that emerge are (1) maximum biomass at each trophic level is controlled from below (bottom-up) by nutrient availability, (2) this bottom-up regulation is strongest at the bottom of the food web (i.e. phosphorus → phytoplankton) and weakens by a factor of 2 with each succeeding step up the food web, (3) as energy moves up a food web, the predictability of bottom-up interactions decreases, (4) near the top of the food web, top-down (predator mediated) interactions are strong and have low coefficients of variation, but weaken with every step down the food web, (5) variability around the bottom-up regressions can always be explained by top-down forces, and (6) interplay between top-down and bottom-up effects changes with the trophic status of lakes. In eutrophic lakes, top-down effects are strong for piscivore → zooplankton, weaker for planktivore → zooplankton, and have little impact for zooplankton → phytoplankton. For oligotrophic lakes, the model predicts that top-down effects are not strongly buffered, so that zooplankton → phytoplankton interactions are significant.

Les incidences relatives des forces « ascendantes » (régies par les producteurs) et « descendantes » (régies par les consommateurs) sur la biomasse et la structure des tailles de cinq importants groupes ou paramètres des systèmes pélagiques d'eau douce (piscivores, planctonivores, zooplancton, phytoplancton et disponibilité du phosphore total) ont fait l'objet d'une modélisation. Selon ce modèle : (1) la biomasse maximale de chaque niveau trophique est régie par la disponibilité des matières nutritives des niveaux inférieurs; (2) cette régulation de type ascendant est la plus marquée à la partie inférieure de la chaîne alimentaire (phosphore → phytoplancton) et s'amoindrit par un facteur de 2 à chaque étape; (3) la possibilité de prévoir les interactions dans le sens ascendant diminue à mesure que l'énergie remonte la chaîne alimentaire; (4) près de la partie supérieure de la chaîne, les interactions vers le bas (par l'intermédiaire des prédateurs) sont importantes et de faible coefficient de variation, mais elles s'amenuisent avec chaque palier inférieur de la chaîne; (5) la variabilité annexe aux régressions ascendantes peut toujours s'expliquer par les forces agissant vers le bas; et (6) les relations entre les effets vers le bas et vers le haut se modifient en fonction de l'état trophique du lac. Dans les lacs eutrophes, les effets descendants sont importants pour la relation piscivores → zooplancton, moins importants pour la relation planctonivores → zooplancton et ont peu d'effets sur la relation zooplancton → phytoplancton. Dans les lacs oligotrophes, les effets descendants ne sont pas fortement tamponnés, de sorte que les interactions zooplancton → phytoplancton sont appréciables.

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The limnological literature that deals with the biomass and abundance of organisms in pelagic food webs supports two apparently contradictory points of view. The first asserts that pelagic trophic level biomass is controlled from below by producers, and the second supports the conclusion that it is controlled from above by consumers. This is reminiscent of a general controversy that has long been part of the terrestrial literature (Hairston et al. 1960; Slobodkin et al. 1967; van Valen 1973) where at one extreme there are those (summarized by White 1978) who contend that biomass at all trophic levels is regulated by resource quantity and quality, and at the other extreme (exemplified by Mech et al. 1971; Simenstad et al. 1978) there are those who champion control by consumers (predators).

To date, the terrestrial literature has not yielded a concensus, because good empirical evidence has been extremely difficult to acquire; however, empirical data are now available for freshwater pelagic systems and support both the top-down and bottom-up hypotheses.

The evidence supporting the "producer-controlled" (bottom-up) model comprises empirically derived relationships based on data from many lakes. The relationships take the form of regression lines plotting the biomass of the dependent variable (consumer) as a function of the independent variable (producer). There are four basic sets of regressions that apply to freshwater pelagic ecosystems. The first is the relationship between total phosphorus (independent variable) and chlorophyll *a* (Sakamoto 1966; Dillon and Rigler 1974; Schindler

TABLE 1. Summary of top-down regression lines. ZB = zooplankton biomass, PLB = planktivore biomass, CHLA = chlorophyll *a*, PHB = phytoplankton biomass, TP = total epilimnetic phosphorus, DAP > 1 = *Daphnia* > 1 mm, *n* = sample size, r^2 = coefficient of variation, ww = wet weight, dw = dry weight. An asterisk indicates a model II regression; all others are model I regressions.

Regression	*n*	r^2	Comments
Planktivores and zooplankton			
(1)* ZB ($mg \cdot L^{-1}$ ww) = 2.667 − 1.834PLB ($mg \cdot L^{-1}$ ww)	53	0.78	Each *n* represents one weekly sample (July, Aug., Sept. 1981)
(2) ZB ($mg \cdot L^{-1}$ ww) = 7.89 − 2.09PLB ($mg \cdot L^{-1}$ ww)	4	0.96	Each *n* is the average of eight weekly samples (May, June 1983)
(3)* $\log_{10}$ ZB ($\mu g \cdot L^{-1}$ dw) = 2.69 − 0.43 $\log_{10}$ PLB ($\mu g \cdot L^{-1}$ ww)	53	0.59	As above, all fish data
Zooplankton and phytoplankton (chlorophyll a*)*			
(4) $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 2.27 − 1.08 $\log_{10}$ ZB ($mg \cdot L^{-1}$ ww)	18	0.21	Each *n* represents one weekly sample (May, June 1981) (Fig. 2)
(5) $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.21 − 0.12 $\log_{10}$ ZB ($mg \cdot L^{-1}$ ww)	23	0.01	Each *n* represents one weekly sample (May, June 1983) (Fig. 2)
(6)* $\log_{10}$ PHB ($\mu g \cdot L^{-1}$ ww) = 7.82 − 1.96 $\log_{10}$ ZB ($\mu g \cdot L^{-1}$ ww)	54	0.07	Each *n* represents one weekly sample (July, Aug., Sept. 1982)
Cladocera ≥ 1 mm length and phytoplankton			
(7) $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.859 − 0.367 $\log_{10}$ DAP > 1 ($\mu g \cdot L^{-1}$ ww)	18	0.65	Each *n* represents one weekly sample, 1981 and 1983. May, June, only non-zero *Daphnia* samples are included
(8) $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.52 − 0.367 $\log_{10}$ DAP > 1 ($\mu g \cdot L^{-1}$ dw)	18	0.64	Each *n* represents one weekly sample, 1981 and 1983. May, June, only non-zero *Daphnia* samples are included
Phytoplankton and mean epilimnetic TP			
(9) $\log_{10}$ TP ($\mu g \cdot L^{-1}$) = 1.774 + 0.250 $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$)	20	0.55	Each *n* represents one weekly sample (May, June 1981) (Fig. 4)
(10) $\log_{10}$ TP ($\mu g \cdot L^{-1}$) = 1.758 − 0.090 $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$)	30	0.02	Each *n* represents one weekly sample (May, June 1983) (Fig. 4)

1978; Janus and Vollenweider 1981; Hanson and Peters 1984; Pace 1984), the second relates phytoplankton biomass or chlorophyll *a* (independent variable) and zooplankton biomass (McCauley and Kalff 1981; Mills and Schiavone 1982; Hanson and Peters 1984; Canfield and Watkins 1984), the third relates zooplankton biomass (independent variable) and planktivore biomass (Mills and Schiavone 1982), and the fourth relates an index of planktivore biomass (independent variable) and an index of piscivore biomass (Mills and Schiavone 1982). When combined, these relationships suggest that the biomass of each of the major trophic levels (phytoplankton, zooplankton, planktivores, and piscivores) is resource limited, i.e. by the producer level immediately below.

The evidence supporting the "consumer-controlled" (top-down) model has spawned the theories of trophic "biomanipulation" (Shapiro et al. 1982; Shapiro and Wright 1984) and "cascading trophic interactions" (Carpenter et al. 1985). At the top of the food web is the negative relationship between piscivores (independent variable) and planktivores (Bonar 1977; Holčík 1977). One step down is the negative relationship between plantivore biomass (independent variable) and zooplankton biomass and species composition (Hrbáček 1962; Brooks and Dodson 1965; Galbraith 1967; Hall et al. 1970; Hutchinson 1971; Stenson 1972, 1976; O'Brien and de Noyelles 1974; Anderson et al. 1978). This relationship is almost entirely unquantified because most studies are based only on relative measures of fish biomass, but recent work (Mills and Forney 1983; Post 1984; McQueen and Post 1984; Vijverberg and van Densen 1984) demonstrates negative relationships which are statistically significant ($p < 0.05$). Finally, the literature also shows that near the bottom of the food web, zooplankton (independent variable) can have a negative impact on phytoplankton biomass (Uhlman 1961; Burns 1968, 1969; Hurlburt et al. 1972; Porter 1977; Lynch 1979; Lynch and Shapiro 1981; Shapiro et al. 1982; Shapiro and Wright 1984; McCauley and Kalff 1981). Taken together, these data suggest that the biomass at each trophic level can be controlled from above by consumers.

In the analysis that follows, we will first present a series of empirically derived regressions which quantify top-down effects. These relationships are based on eutrophic lake enclosure experiments conducted at Lake St. George. The enclosures used are large enough (750 m^3) and deep enough (15 m) to maintain normally growing fish populations for >18 mo (McQueen and Post 1984) and to maintain zooplankton and phytoplankton populations that are similar in terms of numbers and diversity to the communities found growing naturally in Lake St. George (Story 1982). These enclosures have allowed us to manipulate planktivore populations (0+ and 1+ *Perca flavescens* and 1+ *Notemigonus crysoleucas*) and then to monitor the subsequent effects on zooplankton, phytoplankton, and macronutrients. Our second step will be to review the literature and to derive average bottom-up regression lines which relate consumers (dependent variable) to producers (independent

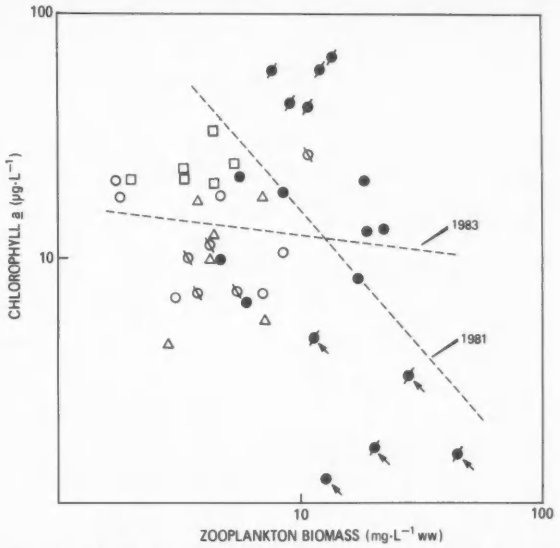

FIG. 1. Chlorophyll *a* biomass plotted with respect to total zooplankton biomass (ww = wet weight). The solid symbols represent 1981 data and the open symbols 1983 data. The 1981 and 1983 lines are given as lines 4 and 5 in Table 1. Arrows denote data points derived from samples containing more than 10% biomass of *Daphnia* >1 mm length. Enclosures described in McQueen and Post (1984) are denoted as follows: 1 = ●, 2 = ●, 3 = ○, 4 = ◔, 5 = △, 6 = □.

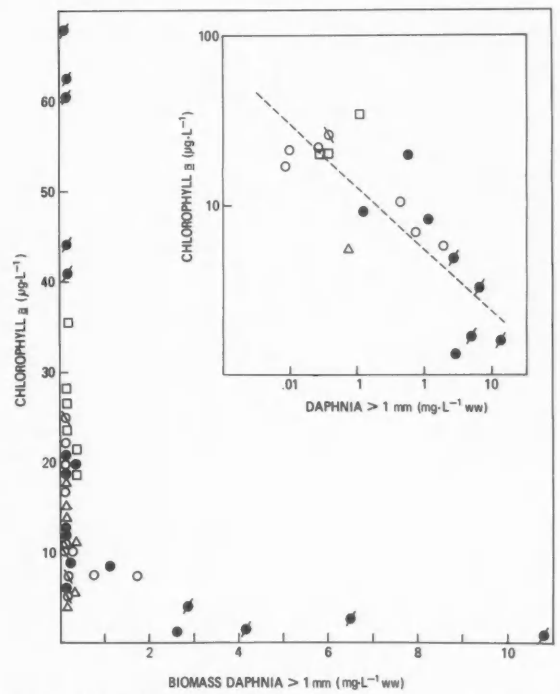

FIG. 2. Chlorophyll *a* biomass plotted with respect to the biomass of *Daphnia* > 1 mm in length (ww = wet weight). Enclosures described in McQueen and Post (1984) are denoted as follows: 1 = ●, 2 = ●, 3 = ◔, 4 = ○, 5 = △, 6 = □. Inset: $\log_{10}$:$\log_{10}$ plot. The line is given as line 7 in Table 1.

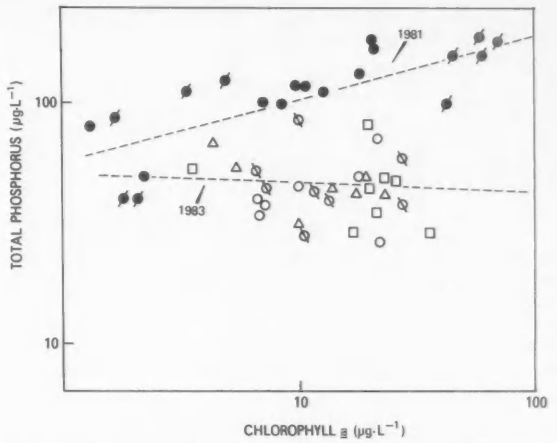

FIG. 3. Total phosphorus plotted with respect to chlorophyll *a*. Enclosures described in McQueen and Post (1984) are denoted as follows: 1 = ●, 2 = ●, 3 = ○, 4 = ◔, 5 = △, 6 = □. The 1981 and 1983 lines are given as lines 9 and 10 in Table 1.

variable). The third step will be to test the hypothesis that bottom-up processes determine maximum biomasses at all trophic levels and that top-down processes are responsible for the observed deviations from each of the predicted relationships. The final step will be to estimate the impact of bottom-up and top-down forces at each level in the trophic food web and to investigate the effects of altered productivity levels.

Methods

Data for the top-down model were derived from enclosure experiments that were run during the spring (May and June) of 1981 and 1983 and the summer (July, August, and September) of 1982. The enclosures measured 8 m in diameter by 15 m deep and were open to the sediment–water interface. The same protocol was used for all the experiments. At ice-out, divers ensured that the weighted enclosure skirts were firmly fixed into the sediments. A high-capacity pump was used to add a minimum of 700 m^3 to each enclosure. During 1981, nutrients (10 g of 90% H_3PO_4 and 250 g of $NaNO_3$) were added to the epilimnion (1 m depth) on a weekly basis, and during 1982 and 1983 half of that amount was added. In the 1981 experiments, water samples were taken at 1, 3, 5, 8, and 12 m, and in the 1983 experiments, samples were taken at 2, 6, and 10 m. Samples were processed at the Canada Centre for Inland Waters using the methods described by Stainton et al. (1977). The mean epilimnetic total phosphorus (TP) concentrations were 118 and 113 $\mu g \cdot L^{-1}$ (two enclosures, 1981) and 45, 49, 56, and 47 $\mu g \cdot L^{-1}$ (four enclosures, 1983). TP was not measured during 1982.

Zooplankton were captured with a vertical haul net pulled between 0 and 12 m. During 1981 the mesh size used was 80 μm, and during 1982 and 1983 the mesh size was 150 μm. The count groups were nauplii, rotifers (excluding *Asplanchna*), *Asplanchna*, *Daphnia*, *Chydorus*, *Diaphanosoma*, *Bosmina*, *Skistodiaptomus oregonensis*, *Ceriodaphnia*, and cyclopoids. Counts were done at 25× or 50× magnification through a Wild stereo microscope. Subsample sizes were adjusted so that all major groups were represented by 40–500 individual counts. During the counts, samples were

TABLE 2. Summary of published bottom-up regression relationships. n = sample size, error mean square. Regressions noted with an asterisk were calculated from published ta SO = spring overturn data, SM = summer mean, WS = weekly samples, dw = dry catch per unit effort, ps = pumpkin seed, bg = bluegill, p = perch, bc = black crappie, C planktivore biomass, PISB = piscivore biomass.

	Regression	n	r^2
			Phosphorus
(1)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.583 $\log_{10}$ P ($\mu g \cdot L^{-1}$) − 1.34	30	0.97
(2)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.449 $\log_{10}$ P ($\mu g \cdot L^{-1}$) − 1.136	46	0.95
(3)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.213 $\log_{10}$ TP ($\mu g \cdot L^{-1}$) − 0.848	81	0.88
(4)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.119 $\log_{10}$ TP ($\mu g \cdot L^{-1}$) − 0.676	29	0.67
(5)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.250 $\log_{10}$ TP ($\mu g \cdot L^{-1}$) − 0.676	49	0.66
(6)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.050 $\log_{10}$ TP ($\mu g \cdot L^{-1}$) − 0.530	57	0.76
(7)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.090 $\log_{10}$ TP ($\mu g \cdot L^{-1}$) − 0.560	12	0.93
(8)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 0.859 $\log_{10}$ TP ($\mu g \cdot L^{-1}$) − 0.446	140	0.7
(9)	$\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) = 1.010 $\log_{10}$ TP ($\mu g \cdot L^{-1}$) − 0.180	13	0.81
			Phytoplankto
(10)	$\log_{10}$ ZB ($\mu g \cdot L^{-1}$ dw) = 0.53 $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) + 1.44	49	0.57
(11)	$\log_{10}$ ZB ($\mu g \cdot L^{-1}$ ww) = 0.511 $\log_{10}$ PB ($\mu g \cdot L^{-1}$ ww) + 1.807	207	0.63
(12)	$\log_{10}$ ZB ($\mu g \cdot L^{-1}$ ww) = 0.719 $\log_{10}$ BP ($\mu g \cdot L^{-1}$ ww) + 1.015	17	0.86
(13)*	$\log_{10}$ ZB ($\mu g \cdot L^{-1}$ dw) = 0.55 $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) + 1.92	12	0.81
(14)*	$\log_{10}$ ZB ($\mu g \cdot L^{-1}$ dw) = 0.59 $\log_{10}$ CHLA ($\mu g \cdot L^{-1}$) + 1.355	11	0.37
(15)	$\log_{10}$ ZN (no. $\cdot m^{-3}$) = 0.50 $\log_{10}$ CHLA ($mg \cdot m^{-3}$) + 4.7	165	0.43
			Zooplankton
(16)*	$\log_{10}$ PLB (index) = 0.465 $\log_{10}$ ZB ($\mu g \cdot L^{-1}$ dw) + 0.893	10	0.24
(17)*	$\log_{10}$ PLB (index) = 0.159 $\log_{10}$ ZB ($\mu g \cdot L^{-1}$ dw) + 2.10	11	0.08
			Planktivore
(18)*	$\log_{10}$ PISB (index) = −0.395 $\log_{10}$ PLB (index) + 3.733	10	0.74
(19)*	$\log_{10}$ PISB (index) = −0.143 $\log_{10}$ PLB (index) + 3.025	10	0.28

le size, r^2 = coefficient of determination, sb = standard error of the slope, S^2yx = lished tables; all others were calculated by the original authors. MA = mean annual data, = dry weight, ww = wet weight, SS = single samples, index = biomass index based on appie, CHLA = chlorophyll *a*, TP = total phosphorus, ZB = zooplankton biomass, PLB =

	r^2	sb	S^2yx	Source(s)	Comments
phorus and phytoplankton					
)	0.975	—	—	Dillon and Rigler 1974; Sakamoto 1966	CHLA = SM, TP = SO
5	0.950	—	—	Dillon and Rigler 1974	CHLA = SM, TP = SO
1	0.880	—	—	Schindler 1978	CHLA = MA, TP = MA
)	0.670	—	—	Prepas and Trew 1983	CHLA = SM, TP = SO
)	0.660	0.113	—	Hanson and Peters 1984	CHLA = SM, TP = SM
7	0.760	—	—	Pace 1984	CHLA = WS, TP = WS
2	0.93	—	—	Pace 1984	CHLA = SM, TP = SM
)	0.77	0.040	0.066	Janus and Vollenweider 1981	CHLA = MA, TP = MA
3	0.81	—	—	Stockner and Shortreed 1985	CHLA = SM, TP = SO
oplankton and zooplankton					
9	0.57	0.064	—	Hanson and Peters 1984	ZB = SM, CHLA = SM
7	0.63	0.044	0.45	McCauley and Kalff 1981	ZB = SS, CHLA = SS
7	0.86	0.074	0.271	McCauley and Kalff 1981	ZB = SM, CHLA = SM
2	0.810	0.084	0.259	Pace 1984	ZB = MA, CHLA = MA
1	0.377	—	0.207	Mills and Schiavone 1982	ZB = SM, CHLA = SM
5	0.43	—	—	Canfield and Watkins 1984	ZB = SS, CHLA = SS
plankton and planktivores					
)	0.24	0.276	0.398	Mills and Schiavone 1982	Data listed in Table 6
1	0.08	0.179	0.258	Mills and Schiavone 1982	Table 5; ps, bg, p, bc
nktivores and piscivores					
)	0.74	0.082	0.064	Mills and Schiavone 1982	Planktivores from Table 5; ps, bg, p, bc
)	0.28	0.081	0.107	Mills and Schiavone 1982	Planktivores from Table 6

suspended in a circular counting chamber (Ward 1955) and projected on a television screen using a microscope-mounted Hitachi television camera. A caliper system (Sprules et al. 1981) was used to measure the length of every individual that was counted. This information was automatically recorded on a PET computer which converted lengths to biomasses using species-specific algorithms (W. G. Sprules, unpubl.) that had been stored as software. Knowing mesh size, net size, haul distance, and either species or group counts and lengths, the computer produced count group-specific length–frequency and weight–frequency tables and group-specific counts per litre and biomass (milligrams per litre).

During 1982, young of the year yellow perch (*P. flavescens*) were seined from Lake St. George and were stocked in densities of 600 (enclosure 1), 200 (enclosure 2), and none (enclosure 3). Plexiglas fish traps (Casselman and Harvey 1973), standard commercial minnow traps (40 × 25 cm), and Windermere traps (90 × 57 cm) were fished in the enclosures at 12 intervals during the experiment. Captured fish were measured for total length and weight and returned. During August, a multiple census mark–recapture experiment was conducted in the enclosures containing fish and those results were combined with weekly catch per unit effort data to produce estimates of fish biomass and numbers.

The relationships used for the development of the producer-controlled model were obtained directly from the literature or were calculated from published data. Conversions from wet to dry weight assumed a conversion ratio of 8:1 (Taguchi and Fukuchi 1975; McCauley and Kalff 1981).

Results and Discussion

Top-down Control

Two data sets yield regressions relating the effects of planktivores on zooplankton (Table 1). The first is based on 53 weekly samples taken from three enclosures and yields a negative slope (Table 1, line 1) which is statistically significant ($r^2 = 0.78$, $n = 53$, $p \ll 0.05$). This relationship is supported by the results of a separate experiment (involving four enclosures) which showed that the relationship between zooplankton biomass and an index of fish biomass is also linear negative and significant ($p \ll 0.05$) (Table 1, line 2).

The effects of zooplankton biomass on phytoplankton biomass were measured in three experiments involving nine enclosures (May and June 1981, $n = 2$; May and June 1983, $n = 4$; July, August, and September 1982, $n = 3$). The 1983 experiment (Fig. 1; Table 1, line 5) and the 1982 experiment (Table 1, line 6) both yield weak negative slopes (not different from zero) and r^2 values (0.1 and 0.07) which are not significant ($p \gg 0.05$). However, the 1981 experiment yielded a relationship (Fig. 1; Table 1, line 4) that was significant ($r^2 = 0.21$, $n = 18$, $p \ll 0.05$). Analysis of zooplankton size and species composition indicated that in the first two cases, large *Daphnia* (functionally defined as *Daphnia* > 1 mm, helmet to base of spine) were never abundant, but that in the third case, *Daphnia galeata mendotae* > 1 mm comprised more than 10% of the zooplankton biomass on five of the sampling weeks. These points are identified in Fig. 2 and suggest that the presence of these large cladocerans was associated with reduced phytoplankton biomass (measured as chlorophyll *a*). When these five points are removed from the 1981 data set the slope and r^2 are no longer statistically significant.

A linear plot of the spring data for chlorophyll *a* and large *D. galeata mendotae* (Fig. 2) suggests that when the biomass of large *Daphnia* is greater than 0.5 $mg \cdot L^{-1}$ wet weight (ww) (0.06 $mg \cdot L^{-1}$ dry weight (dw)), chlorophyll *a* is consistently low, but when large *Daphnia* are absent, chlorophyll *a* values are unpredictable. A plot of chlorophyll *a* and biomass of large *Daphnia* (Fig. 2, inset) yields a significant (Table 1, line 7, $r^2 = 0.64$, $n = 18$, $p \ll 0.05$) negative relationship. The conclusion is that in eutrophic lakes, when large *Daphnia* (or perhaps this could be generalized to large cladocera) are absent, zooplankton cannot reduce phytoplankton biomass, but that when large *Daphnia* are present, a significant inverse relationship exists between large *Daphnia* biomass and phytoplankton biomass.

The relationship between phytoplankton (independent) and epilimnetic TP (Fig. 3) was monitored in two experiments (May and June 1981 and May and June 1983) (Table 1, lines 9 and 10). In one case there was no significant relationship, and in the other the slope was positive. The suggestion is that increased phytoplankton had no measurable impact on TP and that what little impact they might have had was obscured by the strong bottom-up relationship between TP and chlorophyll *a* biomass.

Bottom-up Control

Four groups of published regressions relate the biomass of pelagic phytoplankton, zooplankton, planktivores, and piscivores to the producer level one step down in the trophic food web. The relationship between TP (independent variable) and chlorophyll *a* has received the most attention in the literature (Nicholls and Dillon 1978) and is summarized in Table 2. Although the data sets deal with different lakes representing a variety of productivity levels, they all yield highly significant correlations and show some agreement with respect to slope. The relationship between phytoplankton biomass (usually chlorophyll *a*) and zooplankton biomass or numbers has also been documented in a number of published studies (Table 2), and again the regressions are statistically significant, although the r^2 values are lower than for TP and phytoplankton. The relationship between zooplankton biomass (independent variable) and planktivores (represented by an index of catch per unit effort) has been documented by Mills and Schiavone (1982), and regressions (Table 2) calculated from their original tabular values were either marginally significant or nonsignificant, which suggests that bottom-up control is weak. Finally, regressions of planktivore biomass indices (independent variable) and indices of piscivore biomass based on data published by Mills and Schiavone (1982) (Table 2) yield significant *negative* correlations, which suggests that the bottom-up effect of planktivore abundance on piscivore biomass has a weaker effect than the top-down effect of piscivores on planktivores.

The Model: Bottom-up vs. Top-down

The top-down and bottom-up models are supported by two very different data sets. The top-down data result from manipulations of predator populations that receive equal nutrient inputs, and the bottom-up regressions result from many measurements taken from different lakes that are all assumed to receive stable but different nutrient inputs. To investigate the interactions that are implied by these regressions, each of the four top-down and bottom-up relationships must first be represented by single "best-fit" (Fig. 4) regression lines. The four mean top-down lines follow directly from the experimentally derived data presented earlier. The mean effect of planktivo-

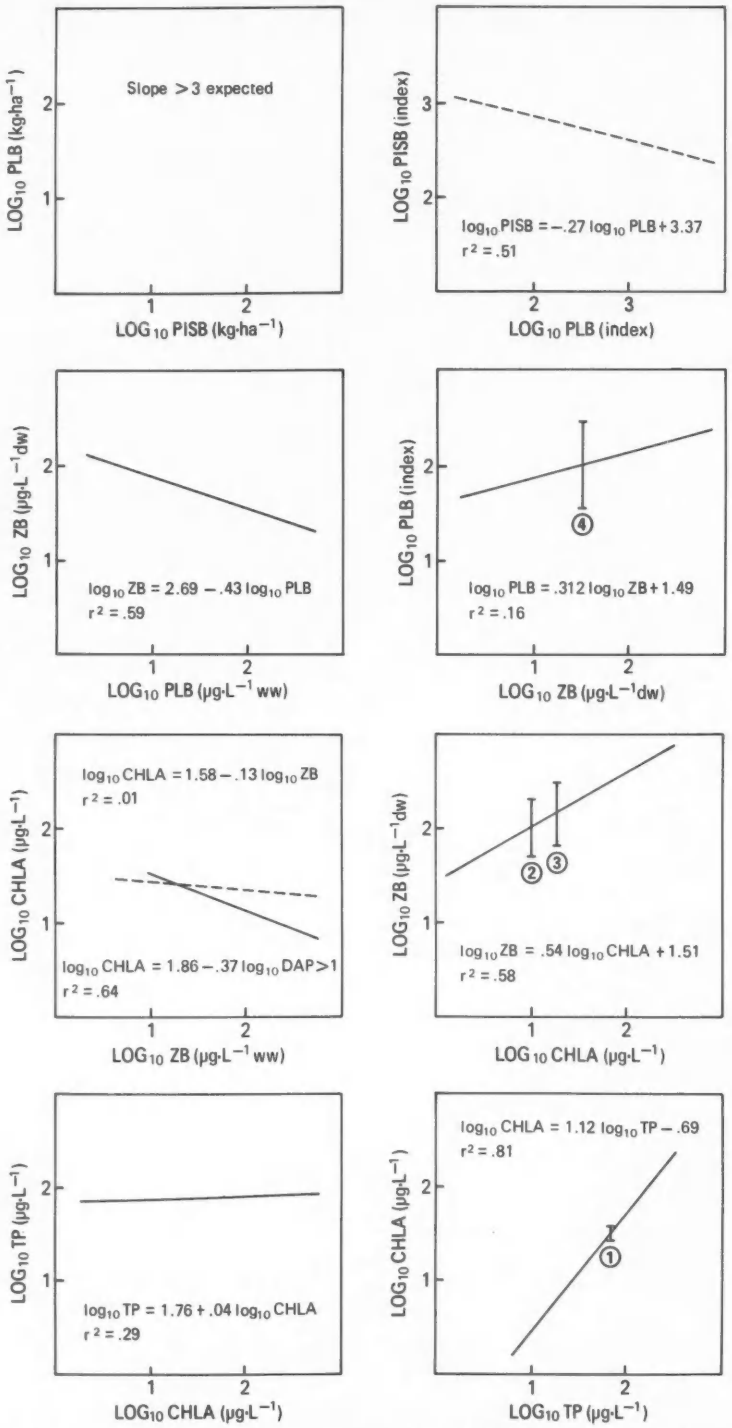

FIG. 4. Mean regression lines for bottom-up relationships (right-hand panels) and top-down relationships (left-hand panels). Numbered 95% confidence intervals are based on data provided by (1) Janus and Vollenweider (1981), (2) and (4) Mills and Schiavone (1982), and (3) Pace (1984). Slopes of the broken lines are not statistically different ($\alpha = 0.05$) from zero.

rous fish on zooplankton has the strongest negative slope and is statistically significant (Table 1, line 3). The effect of total zooplankton biomass on chlorophyll *a* (Table 1, lines 5 and 6) has a slope that is not different from zero and is not statistically significant; however, the effect of large *Daphnia* (Table 1, line 15) on phytoplankton biomass has a significant negative slope. Finally, the relationship between chlorophyll *a* and epilimnetic TP (Fig. 4, *n* weighted slope, intercept, and r^2 based on lines 9 and 10, Table 1) is not significant.

Average values for the producer-controlled regressions have been calculated from published relationships (Table 2). The slopes, intercepts, and r^2 values of the regressions linking TP and chlorophyll *a* were pooled with respect to sample size to produce the relationship shown in Fig. 4. The slopes and intercepts of the regressions linking chlorophyll *a* and zooplankton biomass were pooled with respect to the standard error of the slope, and the r^2 valeus were pooled with respect to sample size (Fig. 4). The same procedure was used to average the lines relating zooplankton biomass to planktivore biomass and planktivore biomass to piscivore biomass.

Inspection of the averaged regressions (Fig. 4) and the original data sets (Table 2) suggests that producer-controlled (bottom-up) regressions have two characteristics that tend to order the data set: (1) slopes are strong near the bottom of the food web and weak near the top and (2) explained variance (r^2) is large at the bottom and small at the top.

These generalizations require cautious analysis because slopes and r^2 could be influenced by the range over which mesurements are made. Fortunately, however, the data (Table 2) suggest that for most regressions, the *X* and *Y* variables have similar ranges. For example, the total TP versus chlorophyll *a* relationship has an average slope of +1.26, and the range for both TP and chlorophyll *a* extends from approximately 2 to 500 $\mu g \cdot L^{-1}$. Similarly, the phytoplankton biomass versus zooplankton biomass regression of McCauley and Kalff (1981) has a slope of +0.511, and the data sets range from approximately 30 to 20 000 $\mu g \cdot L^{-1}$ (fresh weight) for both zooplankton and phytoplankton. Because the range of TP equals the range of chlorophyll *a* and the range of planktivore biomass equals the range of zooplankton biomass, the slopes of the two lines can be compared, and such a comparison suggests that the effect of TP on chlorophyll *a* is more than twice the effect of chlorophyll *a* on zooplankton biomass. Similarly, the effect of chlorophyll *a* on zooplankton biomass is approximately twice as great as the effect of zooplankton biomass on planktivore biomass.

The second unifying characteristic that emerges from the plot of averaged lines is that the variability around the bottom-up regression lines increases by the power of approximately 3.3 (i.e. $r^2 = 0.84^{3.3} = 0.56$, $r^2 = 0.54^{3.3} = 0.13$) (Fig. 4) with every step up the food chain. This, combined with the fact that the variability and slope of the top-down lines (Fig. 4) shows the opposite trend, suggests that perhaps much of the variability in the bottom-up relationships can be explained by top-down effects. For example, the relationship between TP (independent variable) and chlorophyll *a* has variability that could perhaps be explained by zooplankton biomass. Similarly, the relationship between chlorophyll *a* (independent) and zooplankton biomass could perhaps be explained by planktivore biomass, and so on. In the following analysis, we have investigated these possibilities by comparing 95% confidence intervals for each of the average bottom-up regressions with the slopes and ranges of the top-down relationships and estimating the proportion of unexplained variability that could be explained by the trophic level above (consumer or top-down control).

Beginning at the bottom of the food web, the variation around a chlorophyll *a* biomass of 31.6 is 28.0−35.5 $\mu g \cdot L^{-1}$ (95% confidence interval) based on $n = 140$ OECD and Canadian lakes (Janus and Vollenweider 1981). The next trophic level is zooplankton biomass, and the top-down data show that the relationship between zooplankton biomass and chlorophyll *a* is weak and that the slope is not significantly different from zero. It must be concluded, therefore, that zooplankton biomass does not influence chlorophyll *a* in any predictable way and cannot account for any of the variability in the TP − chlorophyll *a* regression. However, the biomass of large *Daphnia* has a surprisingly strong effect on chlorophyll *a* biomass (Fig. 2), and over the range used in the enclosure experiments a change in the biomass of large *Daphnia* from 10 to 1000 $\mu g \cdot L^{-1}$ ww can change chlorophyll *a* from 31.6 to 5.5 $\mu g \cdot L^{-1}$, which is more than enough to explain all of the variability in the TP − chlorophyll *a* regression. The 95% confidence interval derived from the data of Mills and Schiavone (1982) (Table 2) ranges from 48.9 to 204.2 $\mu g \cdot L^{-1}$ dw around a mean zooplankton biomass of 100 $\mu g \cdot L^{-1}$ dw, and the 95% confidence interval based on the data of Pace (1984) ranges from 66 to 295 $\mu g \cdot L^{-1}$ dw (Table 2) around a zooplankton biomass of 141 $\mu g \cdot L^{-1}$ dw. The trophic level above zooplankton is planktivore biomass, and the top-down data (Fig. 4) show that as planktivores range from 0 to 2000 $\mu g \cdot L^{-1}$ (0−100 $kg \cdot ha^{-1}$), zooplankton biomass changes from 700 to 400 $\mu g \cdot L^{-1}$ dw in the spring and from 90 to 25 $\mu g \cdot L^{-1}$ dw in the summer (Post 1984). Such changes are enough to explain almost all of the variability around the empirical chlorophyll *a* − zooplankton biomass regression. Finally, the variability around the zooplankton biomass − planktivore biomass regression (based on Mills and Schiavone 1982) ranges from 35 to 287 planktivore units based on a mean of 100 units. Because there is no top-down regression (Fig. 4) that relates piscivores and planktivores, it cannot be shown that this variability can be explained in terms of piscivore biomass, but the expectation is that the top-down relationship is very strong and will explain the observed variability. This conclusion is supported by the relationship between fish yield (dependent variable) and percentage predatory fish ($r = -0.574$) observed in 43 Polish pikeperch lakes (Bonar 1977).

The Model: Eutrophic and Oligotrophic Systems

The model (Fig. 5) that emerges from this analysis bears some similarity to the HSS model proposed by Hairston et al. (1960) and Slobodkin et al. (1967). Like the HSS model, our treatment (Fig. 5) predicts that producers (chlorophyll *a*) are primarily regulated by nutrient supply and are only influenced by predators (zooplankon biomass) when large *Daphnia* are present. Both models (Fig. 5) also predict that zooplankton are strongly influenced by carnivores (planktivores) although the eutrophic pelagic (EP) model predicts that maximum attainable zooplankton biomasses are set from below and that divergence from this mean is controlled from above. Finally, the HSS model predicts that carnivore biomass is controlled from below. This is, in part, contrary to the predictions of the EP model which suggests that planktivores are strongly influenced by piscivore biomass, and that piscivore regulation is attained through a combination of self-regulation and regulation by resource (prey) availability based on the biomass of benthic organisms and benthivorous fish.

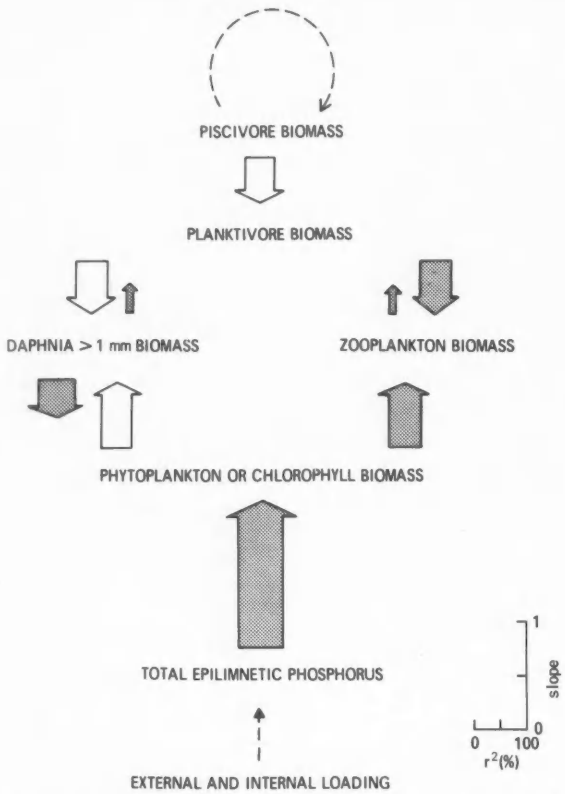

FIG. 5. Flow diagram showing the relative influence of each trophic level on other trophic levels. The length of each arrow represents the magnitude of the effect (slope). The width of each arrow represents the proportion of variability accounted for by the regression (r^2). A scale for r^2 and slope is given in the lower right corner of the figure. The single broken line joining loading and TP is supported by many studies but has not been quantified in this model. The broken line joining piscivores to piscivores is hypothesized to be strong but the relationship cannot yet be supported by empirical data. The open arrow joining piscivores and planktivores represents a *minimum* estimate based on the empirical relationship between planktivores (independent) and piscivores (equations 19 and 20, Table 2). We hypothesize that experimental results will show that the relationship is stronger than indicated. The open arrows joining planktivores to *Daphnia* and phytoplankton to *Daphnia* are equal to the relationships obtained for planktivore biomass and zooplankton biomass and for chlorophyll *a* and zooplankton biomass, i.e. *Daphnia* are assumed to be no different than "average zooplankton." Subsequent analysis will be required before this estimate can be finely tuned.

The test of any model must lie in its ability to predict or perhaps even to explain field observations. In at least two cases the EP model fails to do this. The first involves a study by Yan et al. (1982) which shows that in Mountaintop Lake, there was a strong negative correlation between phytoplankton biomass and the biomass of *Diaptomus minutus* and *Bosmina longirostris*. Since neither are "large *Daphnia*," the EP model predicts that this should be impossible. The second example comes from Stenson et al. (1978) who showed that when roach (*Leuciscus rutilis*) were removed from Lake Lilla Stockelidsvatten, the dominant zooplankter changed from *B. longirostris* to *Eudiaptomus gracilis*, that *Chaoborus* increased, that water clarity improved, and that primary production decreased. Again, without the presence of "large *Daphnia*," the EP model would not make this prediction.

The solution to this disagreement perhaps lies in the fact that both Mountaintop Lake and Lake Lilla Stockelidsvatten are oligotrophic and the EP model is based on top-down data from a eutrophic lake. Support for this possibility comes from the work of Brocksen et al. (1970) and Oksanen et al. (1981). Oksanen et al. (1981) showed that at low productivities, the effect of herbivores on producers (top-down) should be great, but that as productivity increases, the impact of herbivores on plant communities should decrease. In the case of Brocksen et al. (1970), the evidence which supports this model came from three lakes that contained sockeye salmon (*Oncorhynchus nerka*). In Lake Owikeno, productivity was low and the regression between sockeye biomass (independent) and zooplankton biomass was strongly negative. Data from the more productive Lake Babine–Nilkitkwa showed a similar negative relationship and a higher mean level of zooplankton biomass. Data from the most productive lake, Dalnee, showed little effect of fish on zooplankton and the highest zooplankton biomass. Brocksen et al. (1970, fig. 13) speculated that a similar relationship should exist between zooplankton biomass (independent variable) and phytoplankton.

Combining the Brocksen et al. (1970) and Oksanen et al. (1981) models with the EP model produces a more complete treatment (Fig. 6) which predicts that as productivity increases, top-down control at lower trophic levels weakens and that bottom-up control becomes more dominant. The Oksanen et al. (1981) model also suggests that as productivity increases, top carnivore (piscivore) diversity and biomass increases (Svärdson 1976; Arruda 1979). The conclusion is that increased productivity has two effects: (1) to increase biomass at all trophic levels and (2) to decrease the impact of top-down control. In oligotrophic systems, top-down effects are strong at the primary producer and herbivore level, but with increased nutrient loading and production, top-down control moves up the food web (Fig. 6) so that primary producers are only affected by substantial biomasses of large *Daphnia*.

The general conclusions of this analysis are that when the EP model is revised to account for lake productivity, the patterns commonly observed in both oligotrophic and eutrophic systems are consistent with the predictions made by the model. For both classes of lakes the model predicts that (1) maximum attainable trophic level biomasses are set by bottom-up effects (2) the top-down effects of piscivores on planktivores will always be strong, (3) planktivores will have a $\log_{10}$:$\log_{10}$ linear negative impact on zooplankton biomass and (4) a nonlinear production-dependent impact on zooplankton species composition and size selection, and (5) when planktivores are reduced or absent, invertebrate predators (McQueen 1969; Neill and Peacock 1980; Yan et al. 1982) will influence the structure and perhaps the biomass of the zooplankton community. The revised EP model also predicts that (6) in eutrophic lakes, only large-bodied zooplankton are efficient enough to have negative impacts on phytoplankton biomass, but (7) in oligotrophic systems, small-bodied zooplankton (Stenson et al. 1978; Yan et al. 1982) can reduce nutrient-limited phytoplankton populations. Finally, the model predicts that (8) algal biomass has no measureable effect on TP concentrations.

The Model: Implications for Lake Management

The modified EP model makes two predictions that are appli-

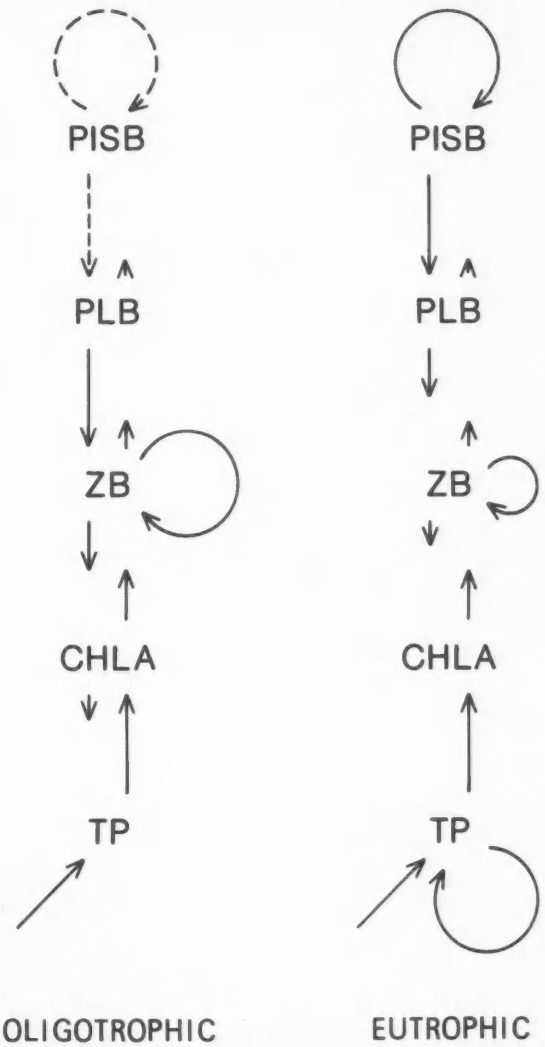

FIG. 6. Comparison of trophic level dynamics in oligotrophic and eutrophic pelagic freshwater systems. The lengths of the arrows indicate the relative importance of the interactions. PISB = piscivore biomass, PLB = planktivore bimass, ZB = zooplankton biomass, CHLA = chlorophyll *a*, and TP = total epilimnetic phosphorus.

cable to biomanipulation and fish enhancement. The first is that increased nutrient additions will have a minimal impact on planktivore production. This prediction derives from the assumption that it is possible to model biomass enhancement using the average bottom-up regression lines (Fig. 4). For example, a 10-fold increase in TP yields a 12-fold increase in chlorophyll *a* which results in a 4-fold increase in zooplankton biomass and a 1.5-fold increase in planktivore biomass. It is important to recognize, however, that such multitrophic level cascading simulations can be misleading. For example, Hanson and Peters (1984) analyzed producer-controlled empirical relationships for 38 lakes and derived relationships for TP − chlorophyll *a*, chlorophyll *a* − zooplankton (crustacean) biomass, and chlorophyll *a* − zooplankton biomass. Using the first two regressions, an increase in TP from 10 to 100 $\mu g \cdot L^{-1}$ increases zooplankton biomass from 55 to 253 $\mu g \cdot L^{-1}$ dw. Using the third relationship, a similar increase in TP increases zooplankton biomass from 46 to 371 $\mu g \cdot L^{-1}$ dw. The lack of agreement between the simulated outcomes could be due to either biological or statistical reasons. The statistical explanation derives from the fact that the standard errors on the slopes of the three regressions are large enough to explain the lack of agreement. The biological explanation involves the existence of alternate pathways (bacteria, protozoa) linking TP and chlorophyll *a*.

A similar analysis applies to the relationship between TP and planktivore biomass. Hanson and Peters (1984) have shown that a 5-fold increase in macrobenthos (dependent variable) is associated with a 10-fold increase in TP, and Hanson and Leggett (1982) have shown that a 3.5-fold increase in fish biomass is associated with a 5-fold increase in macrobenthos (independent variable). Finally, Hanson and Leggett (1982) have shown that a 5-fold increase in fish yield is associated with a 10-fold increase in TP (independent variable). In summary, a 10-fold increase in TP is directly associated with a 5-fold increase in fish yield, with most of the energy moving through the benthic pathway to benthivorous and piscivorous fish. There is also a growing literature which suggests that planktivores are strongly regulated by piscivores. Bonar (1977) showed a negative relationship between percent fish biomass comprising piscivores and total fish yield in 43 Polish pike-perch lakes ($r = -0.574$), and Holčík (1977) working in Klíčava reservoir showed similar negative relationships between percent piscivores and total yield ($r = -0.80$, 1968–72; $r = -0.99$, 1957, 1964, and 1967) and between percent piscivores and yield of forage fish ($r = -0.87$). These relationships all support the conclusion that obligate planktivores are doubly vulnerable. The cascading, bottom-up analysis implies that only about 30% of the potential energy that enters a lake as macronutrients moves directly through the pelagic pathway to planktivores and that about 70% of the potential energy cycles through the macrobenthos to obligate and facultative benthivores. This suggests that the maximum potential biomasses of planktivores should be determined by bottom-up forces, but that realized biomasses should be strongly controlled from above by stable populations of piscivores that can also prey on benthivorous fish and macrobenthos. The management implication is that lake fertilization may increase total fish biomass but will have little effect on planktivore biomass.

The second prediction from the revised EP model is that empirical regressions relating chlorophyll *a* (dependent variable) to epilimnetic TP should include a subset of eutrophic lakes which have chlorophyll *a* values that are much lower than expected and that these lakes should have populations of large *Daphnia* that are maintained either by a refuge or by planktivore biomasses that are less than some production-specific minimum biomass. Some limited support for this prediction comes from Osgood (1983) and Vanni (1983) who observed that large populations of large *Daphnia pulicaria* and *Daphnia pulex* were correlated with low algal biomasses. More convincing evidence comes from Hoyer and Jones (1983) and Pace (1984) who found that only "large zooplankton" biomasses could reduce the sum of squares in TP versus chlorophyll *a* regressions. Many authors have qualitatively shown that the presence of vertebrate predators is associated with reductions in the biomass of large herbivores (Hrbáček 1962; Brooks and

Dodson 1965; Galbraith 1967; Hall et al. 1970; Hutchinson 1971; Stenson 1972, 1976; O'Brien and de Noyelles 1974; Hall et al. 1976; Anderson et al. 1978; de Bernardi and Giussani 1978; Gophen 1979, 1985). Recently, this relationship has been quantified by Mills and Forney (1983) who concluded that daphnid populations could not be sustained at fish populations >20–40 kg·ha^{-1}, by McQueen and Post (1984) who found that *D. galeata mendotae* could not survive at planktivore populations >40kg·ha^{-1}, and by Post (1984) who found that when juvenile perch attained biomasses of 20 kg·ha^{-1}, *D. galeata mendotae* populations declined. Together, these data suggest that when planktivore populations exceed a quantifiable production-dependent biomass, predation rates on large-bodied zooplankton exceed production rates and large-bodied forms are eliminated. This effect will certainly be important in eutrophic systems, but may be less so in oligotrophic lakes where both large and small herbivores can reduce algal standing stocks (Stenson et al. 1978; Yan et al. 1982). This suggests that there will not be a subset of oligotrophic lakes that have chlorophyll *a* concentrations that are lower than expected. The management implication is that in eutrophic lakes where large-bodied zooplankton are required for phytoplankton regulation, managers will have to ensure that production-specific planktivore biomasses are not exceeded. To date, there are no data which will allow us to predict the impact of this strategy on total fish yield, but it is possible that for eutrophic lakes, improved water quality will only be possible when planktivore yields are reduced to unacceptably low levels. In short, the use of biomanipulation for the control of algal standing stocks in eutrophic lakes must be approached with caution.

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Distribution of the Stonefly Nymph *Paragnetina media* (Plecoptera: Perlidae): Influence of Prey, Predators, Current Speed, and Substrate Composition

Blair W. Feltmate, Robert L. Baker, and Philip J. Pointing

Department of Zoology, Erindale College, University of Toronto, Mississauga, Ont. L5L 1C6

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Effects of prey distribution, presence of a predator, current speed, and substrate composition on distribution of the predaceous stonefly nymph *Paragnetina media* (Walker) were examined in a large laboratory stream. Distribution of prey (*Hydropsyche sparna*, *H. bronta*) did not affect distribution of well-fed or non-fed (48 h) nymphs. However, *P. media* distribution was significantly influenced by independent and interactive effects of current speed (0.33–0.41 $m \cdot s^{-1}$) and substrate composition (53.0–110.0 mm in diameter). In the presence of rainbow trout (*Salmo gairdneri*), *P. media* selected more specifically for larger substrate on which risk of predation was less than on smaller size classes. Lighting condition had no effect on substrate selection. Laboratory findings were consistent with distribution of *P. media* observed in the Credit River, Ontario, during midsummer, late fall, and early spring.

Les auteurs ont étudié les effets de la distribution des proies, de la présence d'un prédateur, de la vitesse du courant et de la composition du substrat sur la distribution de nymphes prédatrices du plécoptère *Paragnetina media* (Walker) dans un cours d'eau–laboratoire important. La distribution des proies (*Hydropsyche sparna*, *H. bronta*) n'a pas eu d'effet sur la distribution de nymphes bien alimentées ou non alimentées (48 h). La distribution de *P. media* a cependant été significativement modifiée par les effets indépendants et interactifs de la vitesse du courant (0,33–0,41 $m \cdot s^{-1}$) et de la composition du substrat (granulométrie de 53,0–110,0 mm). En présence de truites arc-en-ciel (*Salmo gairdneri*), *P. media* choisissait spécifiquement les substrats à granulométrie grossière où les risques de prédation étaient moindres. Les conditions d'éclairement n'influaient pas sur le choix du substrat. Les résultats obtenus en laboratoire étaient cohérents avec la distribution de *P. media* observée dans la rivière Credit (Ontario) au cours du milieu de l'été, tard en automne et tôt au printemps.

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Several biotic and abiotic factors may interact to affect distribution of benthic insects in streams. These factors may exert their influence in a homogeneous manner over extended sections of stream (e.g. temperature, water chemistry, dissolved oxygen) or they may be heterogeneous in localized patches (e.g. current velocity, substrate type, detritus conditions) (Minshall 1984). The effects of substrate composition on species distribution have been studied intensely since the 1940's (Wene and Wickliff 1940). Wene and Wickliff introduced the technique of placing substrate-filled trays or baskets in streams and determining which species had colonized the various substrates after a number of days. This methodology has been used extensively to determine independent effects of current and substrate on distribution of nymphs (Minshall and Minshall 1977; Rabeni and Minshall 1977; Reice 1980; Erman and Erman 1984). In these studies, interactive effects of current and substrate on nymphal distribution were not considered.

Recently, manipulative experiments have been used in streams to study the influence of prey distribution on that of predators. Peckarsky and Dodson (1980) found no difference between number of predaceous stoneflies colonizing cages with high prey densities and number colonizing those with zero densities. They suggested that either distribution of predaceous stoneflies in streams may not be influenced by prey distribution or that mutual interference may force some nymphs away from areas of high prey density. Peckarsky and Dodson also postulated that distribution of stonefly nymphs might be affected more by physical factors.

Two physical factors that may be important in influencing distribution of the predaceous stonefly nymph *Paragnetina media* are current speed and substrate composition. In the Credit River, Ontario, this stonefly tends to be associated with relatively large substrate particles in rapidly flowing water. Under typical conditions in streams, substrate belonging to a particular size class can occur within a range of current speeds, or several particle size classes can be associated with a particular flow velocity. Thus, when considering the effects of current and substrate on nymphal distribution, both independent and interactive effects of these factors should be considered.

Predation pressure is another factor that may influence substrate selection by *P. media*. Stein and Magnuson (1976) have shown that lentic freshwater crayfish select specific substrates to avoid predation. However, few attempts have been

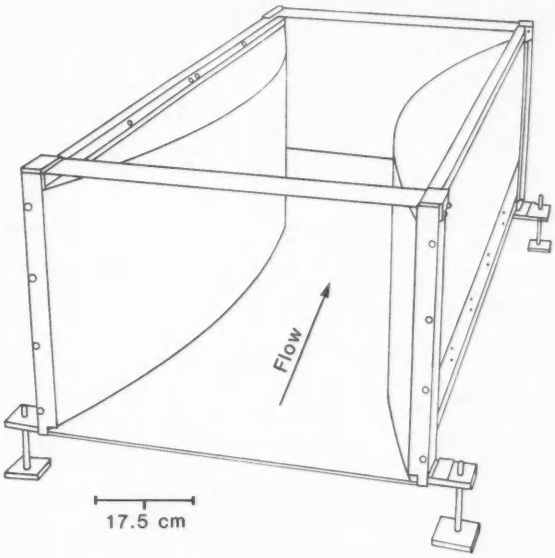

FIG. 1. Current gradient chamber.

made to relate use of substrate by stream invertebrates to risk of predation.

Our objectives in this study were to determine whether prey distribution affects distribution of *P. media* nymphs, to examine effects of current speed and substrate composition on nymphal distribution, and to determine if substrate selection by *P. media* is related to risk of predation by rainbow trout (*Salmo gairdneri*).

General Methods

Field Collections of *Paragnetina media*

We sampled the lower section of the Credit River, Ontario (43°N, 79°W), to determine distribution of *P. media* relative to current speed and substrate composition. At the sampling location the river was approximately 25 m wide and up to 1 m deep. Current speed was measured with an Ott C-1 meter (A. Ott Kempton Company, West Germany) by holding the propeller 2–3 cm above the substrate surface. Current speed ranged from approximately 0.0 to 1.0 $m \cdot s^{-1}$.

Distribution of *P. media* nymphs was determined three times during 1983–84 (midsummer, late fall, and early spring) to monitor possible seasonal changes in distribution. Because low and intermediate flows occur during most days of a typical year commonly observed in lotic systems (Newbury 1984), we sampled a minimum of 5 d after a rainfall. During each period, 50 samples of stonefly nymphs were made using a modified Hess sampler (open area 0.1 m^2, 0.5 m high, 423-μm mesh) at 10 randomly selected points in each of the following current ranges: 0.0–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and >0.8 $m \cdot s^{-1}$ (Hess 1941). Samples of substrate were also collected at each of the points where collections were made. Fine substrate was carried by the current into the collecting sock of the Hess sampler; coarse substrate, not swept into the net, was scooped from the area enclosed by the sampler using a metal bucket. Because stoneflies were not found below 10 cm in the substrate, we did not sample substrate below that depth.

Each substrate collection was dried at 200°C for 3 h which was ample time to remove moisture from the samples. After drying, all samples were sorted through a series of seven sieves into size classes 0.4–1.0, 1.0–2.0, 2.0–4.0, 4.0–11.2, 11.2–22.6, 22.6–53.0, 53.0–110.0, 110.0–250.0, and >250.0 mm.

Laboratory Stream

Experiments were conducted in an oval-shaped laboratory stream in which current speed, water temperature, and photoperiod could be controlled. The two longest sections of the stream were both 6 m in length; however, they were of different widths, one 91 cm and the other 30 cm wide. The size of the stream enabled us to work with relatively large substrate sizes (i.e. up to 110.0 mm in diameter) without substantially reducing current velocity. This stream was useful for experiments involving colonization of substrates by nymphs because the water was circulated by an Archimedes screw through which nymphs passed unharmed. Thus, nymphs could drift from an experimental section in the stream, recirculate, and recolonize the section.

Laboratory experiments were carried out at 21°C which is representative of summer temperatures at the site where *P. media* nymphs were collected. Dechlorinated water was used in all experiments. Dissolved oxygen in the water was 7.9 ppm at 21°C (approximately 90% saturation).

Experimental Methods and Results

Experiment 1: Effects of Prey Distribution on Distribution of *P. media*

To determine if distribution of stonefly nymphs was affected by distribution of prey, we used four pairs of transparent Plexiglas troughs (15 cm deep × 15 cm wide × 90 cm long) placed in the artificial stream. The open-ended troughs were divided across their widths with clear, plastic partitions (2.5 cm high) into four sections (2.5 cm deep × 15 cm wide × 22.5 cm long). Substrate ranging in size from 22.6 to 53.0 mm was placed over the bottom of the trough. Current speed within troughs was 0.3 $m \cdot s^{-1}$. We used *Hydropsyche sparna* and *H. bronta* larvae as prey because we observed *P. media* nymphs consuming hydropsychids readily in the laboratory. Tarter and Krumholz (1971) and Fuller and Stewart (1977) have reported similar observations. The plastic dividers restricted movement of hydropsychids between sections but stoneflies could move throughout the trough. In one member of each pair of troughs, 12 prey were placed in the first and third sections. In the second trough of each pair, we placed 12 larvae in each of the four sections. The size of prey patches was representative of the size observed, qualitatively, in the Credit River, by hand-picking stones; these patches were approximately 15 × 15 cm. In this and all subsequent experiments only large *P. media* nymphs (head width > 3.5 mm) were used. In each trough two stonefly nymphs were released. After a 2-h settling period, the positions of stoneflies were determined visually (without physical disturbance) every 2 h between 13:00 and 19:00 the first day and from 09:00 to 17:00 the second day. Nymphs were on 12 h light : 12 h dark cycle (lights on from 09:00 to 21:00). Previously, we observed *P. media* feeding actively in the laboratory during light hours; thus, we made observations with lights on. If nymphal distribution was influenced by prey density, we would expect to observe *P. media* more frequently in

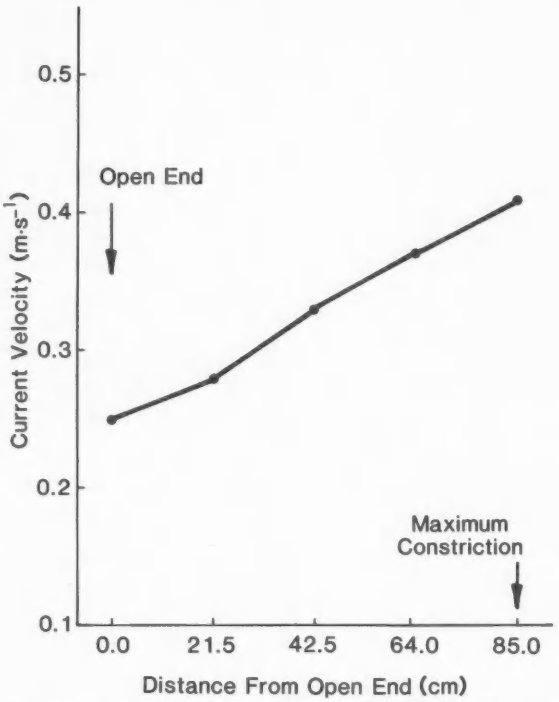

FIG. 2. Relationship between current velocity and distance from the open end to the centre of the current gradient chamber.

prey sections of prey/no prey troughs than in equivalent prey sections in troughs which contained prey in all sections.

Results

Stonefly nymphs, fed and non-fed (48 h), were seen crawling unimpeded between sections within feeding chambers. Caddisfly larvae, however, did not move from the section in which they were originally placed.

Distribution of fed and non-fed nymphs in the four sections of the feeding chambers was not affected by prey distribution (chi-square, $p > 0.10$ and $p > 0.50$, respectively). If stoneflies were influenced by prey distribution more during darkness than light, we would have observed more nymphs in prey patches at the end of the dark photoperiod than during light. No such pattern was observed. In troughs containing fed and non-fed stoneflies, 15–20% of the hydropsychid prey population was depleted over the duration of the experiments.

Experiment 2: Influence of Current Speed and Substrate Composition on Distribution of *P. media*

To control current speed and substrate composition, we used a current gradient chamber (Fig. 1) within which current and substrate could be manipulated. The gradient was created using a venturi within a rectangular chamber; the chamber was placed in the artificial stream, and as water flowed from the upstream opening toward the point of maximum constriction, current speed increased, and towards the downstream end of the venturi, initial flow velocity was resumed. A double, symmetrical gradient was created along the long axis of the chamber (Fig. 2). We observed no measurable side-to-side gradient in the current gradient chamber.

The rectangular, open-ended chamber, within which the venturi was placed, was 170 cm long × 70 cm wide × 50 cm high. The floor of the chamber was made of clear Plexiglas. The walls of the venturi, following a smooth curve, restricted flow through a minimum width of 35 cm at the centre of the chamber. The venturi and Plexiglas floor abutted tightly which prevented animals from escaping. Within the artificial stream, nymphs could drift from the chamber, circulate, and recolonize it from upstream.

Nymphs were collected from the Credit River and kept in an incubator at 21°C on a 12 h light : 12 h dark cycle. Air stones were used to aerate the water in the containers. Each nymph was fed two to three enchytraeid worms per day prior to experiments.

Experiment 2a

To determine if nymphs select current (slow = 0.25–0.33 $m \cdot s^{-1}$, fast = 0.33–0.41 $m \cdot s^{-1}$) or substrate within distinct size class ranges, we placed substrates of four size classes, 11.2–22.6, 22.6–53.0, 53.0–110.0 mm, and a heterogeneous mixture (composed of 25% by weight of substrate in the classes 4.0–11.2, 11.2–22.6, 22.6–53.0, and 53.0–110.0 mm), longitudinally and contiguously in the current gradient chamber. For each of 15 replicates, substrates were arranged in a randomized block pattern. Twenty-five stoneflies were used over 24 h on a 12 h light : 12 h dark cycle. Distribution of nymphs among the four substrate classes within the fast and slow sections of flow was recorded after 24 h. Feltmate (1985) showed that 24 h was ample time for nymphs to select current or substrate and that a density of 25 nymphs per chamber was below the density where intraspecific interactions would affect their distribution.

Experiment 2b

From field collections, we observed a trend towards higher stonefly densities in the current range of 0.2–0.4 $m \cdot s^{-1}$ (Fig. 3). A high density of nymphs within this range might have been influenced by current speed, substrate composition, an interaction between the two, or by some other factor which we were not considering. To determine the relative influence of current speed and substrate composition on nymphal distribution, we released nymphs in the current gradient chamber such that they could select any of four current/substrate combinations. Again, current speed in the chamber ranged from slow (0.25–0.33 $m \cdot s^{-1}$) to fast (0.33–0.41 $m \cdot s^{-1}$). Two substrate compositions, each representative of those found in the Credit River in the current ranges of 0.0–0.2 and 0.2–0.4 $m \cdot s^{-1}$, were placed side by side in the current gradient chamber. If nymphal distribution in the field was influenced by the current/substrate combination which occurred in 0.2–0.4 $m \cdot s^{-1}$ flow, we expected nymphs to select substrate typical of this current velocity within the higher velocity section of the current gradient chamber. The substrate typical in composition to that found in current of 0.0–0.2 $m \cdot s^{-1}$ was chosen as an alternative substrate because it differed markedly in composition from that found typically in the current range of 0.2–0.4 $m \cdot s^{-1}$, and also because *P. media* nymphs were not normally found on this substrate in the Credit River. To make artificial substrate compositions representative of those which occur in the field, appropriate amounts were mixed, by weight, based on field data for rocks in the size classes 4.0–11.2, 11.2–22.6, 22.6–53.0, and 53.0–110.0 mm. We did not use substrate outside of these size ranges because of the physical limitations of our experimental system. Distribution of 25 nymphs, released at random

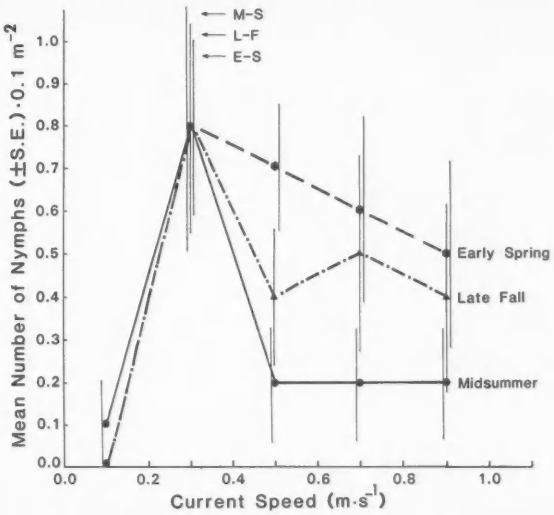

FIG. 3. Distribution of *P. media* nymphs in five current ranges in the Credit River, Ontario, based on 50 collections made with a Hess sampler (0.1 m^2) during each of early spring (E-S), midsummer (M-S), and late fall (L-F).

points in the current gradient chamber, was recorded after 24 h on a 12 h light : 12 h dark cycle. Fifteen replicates were performed.

Results, experiment 2a

Two-way analysis of variance (ANOVA, nonparametric due to lack of normality) (Scheirer et al. 1976) showed interactive ($p \ll 0.001$) and independent effects of current ($p < 0.001$) and substrate ($p < 0.001$) on *P. media* distribution. More nymphs selected substrates in the fast-flow region of the current gradient chamber than in the slow-flow region, and this difference was greatest for the larger and the heterogeneous substrates (Fig. 4).

Results, experiment 2b

Two-way ANOVA indicated strong interactive ($p < 0.001$) and independent effects of current ($p < 0.001$) and substrate ($p < 0.001$) on distribution of *P. media*. Most nymphs were found on substrate typical of that found in the Credit River in the current range of 0.2−0.4 $m \cdot s^{-1}$ when that substrate was placed within the 0.33−0.41 $m \cdot s^{-1}$ section of the current gradient chamber.

Experiment 3: Influence of Predation Risk on Substrate Selection by *P. media*

We used barbless hooks to catch rainbow trout in the Pine River near the town of Alliston, Ontario (44°N, 79°W). Prior to their use in experiments, trout were maintained for 5 d at 17°C in tanks filled with aerated water. They were fed insects collected from the Credit River. Fish were 1+ year class individuals ranging in size from 10 to 17 cm.

Experiment 3a

To determine effects of substrate size on predation risk, we placed substrates of size classes 11.2−22.6, 22.6−53.0, and 53.0−110.0 mm individually in clear Plexiglas troughs (15 cm wide × 90 cm long × 15 cm deep) fitted with 800-μm screens at each end. Bottoms of troughs containing substrate of

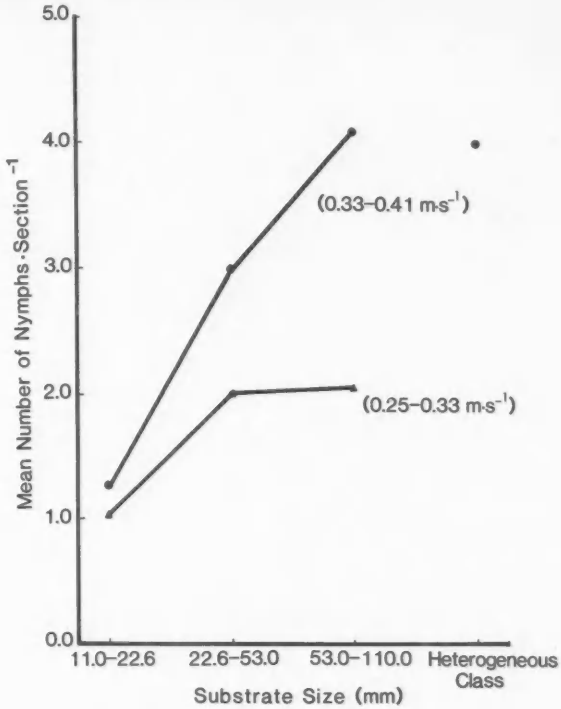

FIG. 4. Interaction of current velocity and substrate composition on the distribution of nymphs of *P. media* in an artificial stream which provided a choice of current velocities and substrate sizes.

size class 11.2−22.6 mm were covered to a depth of two to three stone layers; troughs containing substrate of the other two classes were filled to a depth of one stone layer. We used these depths because they were representative of the field situation. Troughs were arranged in the artificial stream as previously described. Current speed in troughs was 0.30 $m \cdot s^{-1}$ at a point 2−3 cm above the substrate.

We released 10 *P. media* nymphs at random points within each trough 1 h prior to the introduction of fish. Ten nymphs per trough was representative of densities observed at the collection site during midsummer 1985. Two trout were placed in each trough and the experiment was run for 4 d with a 12 h light : 12 h dark photoperiod. After 4 d, we recorded number of nymphs remaining in each trough. This experiment was replicated eight times for small and large substrates and six times for the intermediate class. All replicates were run within 12 d.

Experiment 3b

We examined substrate selection by stoneflies in the presence and absence of fish to determine whether nymphs, in the presence of a predator, select more specifically for substrate on which they are less vulnerable to predation. Each of eight troughs was divided into three areas consisting of an upstream, middle, and downstream section (15 × 30 cm); each area was covered in a different class of substrate. All six possible arrangements of substrates with respect to position in trough were used; two arrangements, chosen at random, were duplicated. To test for substrate selection by stoneflies in the absence of fish, we released 10 stoneflies at random points in

each trough and recorded their position every 12 h over a 24-hr period. Experiments were run using a 12 h light : 12 h dark photocycle to determine if substrate selection was influenced by light levels. Each condition was replicated 32 times.

To test if fish altered substrate selection, we ran experiments identical to those described above except for the presence of one trout per trough. Fish were placed in troughs 10 min after stoneflies. This allowed nymphs to settle but was not long enough for them to select specific substrates prior to introduction of fish (Feltmate 1985).

Results, experiment 3a

The number of *P. media* consumed was related to substrate size (Kruskal–Wallis, $p < 0.001$). Predation was higher on smaller substrate than on intermediate or larger size classes (Dunn's test, Daniel 1978). Our observations suggest that stoneflies were taken more frequently on small substrate because of their increased visibility and accessibility compared with when they occurred on larger substrate. Nymphs on smaller substrate often had body parts protruding above the substrate surface, while those on larger substrates were usually completely hidden. Also, fish could overturn smaller rocks by nudging them with their snout or by fanning substrate with their tail; larger sized substrates were not overturned.

Results, experiment 3b

Two-way ANOVA indicated that the distribution of nymphs was related to presence of fish ($p < 0.001$) but not to light ($p > 0.5$) or the interaction between fish and light ($p > 0.1$). Incidence of *P. media* on smaller substrate was reduced when rainbow trout were present compared with when they were absent (Fig. 5). Changes in the distribution of nymphs in the presence of trout were not due to predation, since all nymphs were recovered from each trough at the end of the experiment.

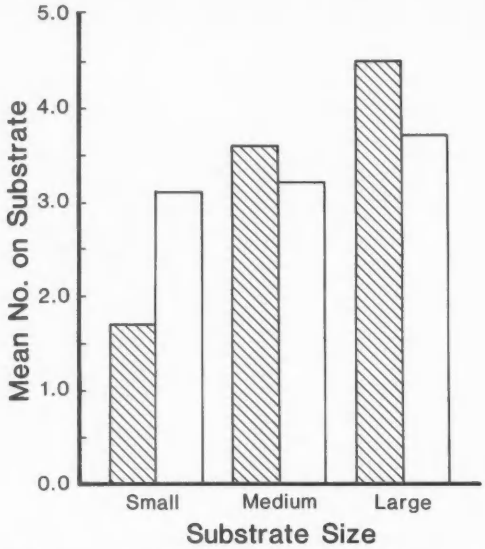

FIG. 5. Mean number of *P. media* nymphs on each type of substrate (small = 11.2–22.6 mm, medium = 22.6–53.0 mm, and large = 53.0–110.0 mm) in the presence (hatched) and absence of rainbow trout.

Discussion

Our finding that distribution of *P. media* nymphs was not affected by distribution of prey is similar to that of Peckarsky and Dodson (1980), who observed no numerical response of stonefly predators to cages that contained different densities of prey. Holling (1961) and Crawley (1975) suggested that such a response is indicative of a predator limited by factors other than prey availability. Peckarsky and Dodson (1980) suggested that mutual interference or physical habitat cues may explain the observed lack of predator response to prey. An unstable or shifting prey patch could also result in no numerical response (Walde and Davies 1984).

Our experiments were designed to preclude the possibility that mutual interference limited predator aggregation in prey patches. Because there were two separate prey patches in each experimental feeding chamber, each of the two predators could select separate sections without interfering with the other.

It is also unlikely that unstable or shifting prey patches affected the observed distribution of *P. media* nymphs. Prey used in these experiments remained in prey sections of feeding chambers and the number of prey per patch was depleted by only 15–20% over the duration of the experiment. Apparently, factors other than prey distribution influence distribution of *P. media*.

Researchers working on lotic systems must consider two or more factors simultaneously to determine if interactions are significant components of insect–substratum relationships (Minshall 1984). Our findings indicate that current and substrate interact to affect distribution of *P. media*. Nymphs select large substrate (53.0–110.0 mm) in fast currents (0.33–0.41 $m \cdot s^{-1}$). Numerous factors may influence this selection.

Substrate samples collected in the field indicated that there was a tendency for more smaller (400 μm – 2.0 mm) particles to be found in the current range of 0.0–0.2 $m \cdot s^{-1}$. Stonefly nymphs which tend to stay in interstitial spaces on the undersurface or sides of rocks would be limited in their ability to crawl under or among substrate found in areas of low flow because particles would fill interstices. In areas of high current velocities, the frequency of occurrence of smaller particles is reduced (Leopold et al. 1964). Thus, in faster flows a stonefly would probably be able to retreat under substrate more readily.

When stoneflies selected the heterogeneous substrate mixture, we observed nymphs on larger substrate (53.0–110.0 mm) more often than on smaller size classes. Selection for larger size substrate was greater whether it occurred in a homogeneous or heterogeneous class. These findings agree with those of Erman and Erman (1984) who found that heterogeneity, defined by the number of size classes, had no effect on colonization by stream invertebrates. Wise and Molles (1979) also found that substrate heterogeneity had no effect on substrate colonization by invertebrates. Allan (1975) found that colonization of mixtures of two to four substrate size classes, by nymphs, was greater than colonization of single size class compositions. In each of these studies, the effect of substrate on colonization was not aimed at specific taxonomic groups. Apparently, to understand the importance of substrate heterogeneity on invertebrate colonization, studies aimed at the genus or species level would be more useful.

Selection by *P. media* of large (53.0–110.0 mm) substrata reduced susceptibility of nymphs to predation by rainbow trout. Similarly, Brusven and Rose (1981) found that various combinations of sand, pebbles, and cobbles significantly influenced predation by a sculpin (*Cottus rhotheus*) on the stonefly

Hesperoperla pacifica, the mayfly *Ephemerella grandis*, and the caddisfly *Rhyacophila vaccua*. Although Brusven and Rose (1981) and Ware (1972) found that the amount and size of substrate affects predation rate, we know of no other studies that suggest that substrate selection by stream invertebrates is altered due to presence or absence of fish. The only related study is that of Stein and Magnuson (1976) who found that, in lentic systems, crayfish selected substrate which afforded more protection from smallmouth bass (*Micropterus dolomieui*).

Similarity in substrate selection by *P. media* under light and no light treatments may be attributed to predation pressure by fish under each condition. Bisson (1978) observed rainbow trout feeding actively during day and night; thus, it is not surprising that *P. media* avoid smaller substrate regardless of lighting.

Our finding that presence of fish alters substrate selection by stoneflies may explain, in part, conflicting reports on substrate selection. For example, differences related to substrate colonization found by Wise and Molles (1979), Erman and Erman (1984), and Allan (1975) may be explained if predation pressure in these streams were considered.

Allan (1982) found that reducing fish biomass to 10–25% of initial values had little effect on prey abundance. He suggested that aquatic insects may be adapted to fish predation and these adaptations may explain the apparent inability of fish to influence abundance of invertebrates in streams. A change in distribution of *P. media* in the presence of rainbow trout may be one means by which this species alleviates predation risk.

Paragnetina media may choose larger substrate because of its increased stability. Small stones will overturn more readily than larger ones as a result of flooding or anchor ice conditions (McAuliffe 1983). McAuliffe found that moss growth on small rocks was less extensive than on large substrates. He attributed this difference in growth to instability of smaller stones. Because large substrates are less likely to move during high discharge, the selection of these substrates by *P. media* may be influenced by the stability of large stones.

In summary, we conclude that prey distribution does not affect that of *P. media*, current speed and substrate composition have an interactive effect on distribution, and that risk due to predation influences substrate selection. Furthermore, we emphasize the need to consider factors simultaneously to detect interactions, and that factors which have been traditionally treated as either biotic or abiotic (e.g. predation pressure, substrate selection) are often related and should not be considered separately.

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New Method for Ageing Chinook Salmon (*Onchorhynchus tshawytscha*) Using Dorsal Fin Rays, and Evidence of Its Validity

D. E. Chilton and H. T. Bilton

Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

Chilton, D. E., and H. T. Bilton. 1986. New method for ageing chinook salmon (*Onchorhynchus tshawytscha*) using dorsal fin rays, and evidence of its validity. Can. J. Fish. Aquat. Sci. 43: 1588–1594.

Age of spawning chinook salmon (*Oncorhynchus tshawytscha*) was estimated using a combination of thin cross-sections from the dorsal fin rays and scales. The latter were used when freshwater growth could not be interpreted from fin rays. Fish of known age were used when available. Results were compared with ages obtained from scales only. Due to scale resorption a significant proportion of the older fish ages was undetected using only scales. Determinations of freshwater age from fin rays and from scales were in agreement 88.5 ± 8.7 to 92.3 ± 7.2% at the 95% confidence level. Thus, the fin ray method provided virtually the same freshwater age as did the scale method. Also, fin rays provided quite acceptable estimation of ocean age. It is recommended that managers use a combination of scales and fin rays to estimate the total age of spawning chinook salmon.

L'âge de géniteurs de saumon quinnat (*Oncorhynchus tshawytscha*) a été estimé par la lecture de coupes minces transversales de rayons de la nageoire dorsale et d'écailles. Ces dernières ont été utilisées lorsque la croissance en eau douce ne pouvait être déterminée à partir des rayons de nageoires. Des poissons d'âge connu ont aussi été utilisés lorsque cela s'est avéré possible. Les âges ainsi déterminés ont été comparés à ceux déterminés à partir d'écailles seulement. La seule lecture des écailles ne permettait pas de déterminer l'âge d'une proportion appréciable des poissons plus âgés à cause de la résorption des écailles. Les nombres d'années passées en eau douce déterminés à partir des rayons de nageoires correspondaient à ceux déterminés à partir des écailles dans de 88,5 ± 8,7 à 92,3 ± 7,2 % des cas, au niveau de confiance de 95 %. La lecture des rayons de nageoires donne donc essentiellement les mêmes résultats que celle des écailles pour la détermination du nombre d'années passées en eau douce. De plus, la lecture des rayons des nageoires permet d'estimer de façon très acceptable le nombre d'années passées en mer. Les auteurs recommandent aux gestionnaires de faire appel à ces deux méthodes pour l'estimation de l'âge des géniteurs de saumon quinnat.

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Accurate age information of chinook salmon (*Oncorhynchus tshawytscha*) caught in the commercial and sport fisheries and escapements is an important part of the vital statistics of one of British Columbia's economically most valuable fish populations. Such information, along with catch and spawning escapement data from different parts of the coast, provides the basis for assessing year-to-year variations in production and effects of stock management.

The ages of chinook salmon caught in a fishery are always estimated by examination of their scales. However, as the salmon reach maturity (on the spawning grounds), most scales are resorbed and total age cannot, therefore, be estimated. At spawning, salmon cease feeding and the scale material is utilized by the fish, resulting in loss of annuli near the periphery of the scale. While ages have been estimated using otoliths in other salmon species (Clutter and Whitesel 1956; Bilton and Jenkinson 1968; Flain 1982), chinook salmon otoliths from stocks along the Pacific coast of Canada are often opaque and therefore unreadable.

Fin rays have previously been used to estimate the age of Pacific salmon species (Bilton and Jenkinson 1969), including sockeye (*O. nerka*) and chum salmon (*O. keta*). However, identification of the freshwater ages proved difficult (Bilton and Jenkinson 1969). Improved techniques in fin ray sectioning, sampling, and preservation suggest that it may now be possible to distinguish freshwater annuli on the fin rays of chinook salmon. We suspect, however, that there may be some deterioration of the fin rays over time among the chinook spawners. Samples taken from hatchery brood stock fish at the time of egg-take appear to be of better quality than those taken after die-off on the spawning grounds. Moreover, the readability of the growth pattern of fin rays that are frozen when stored is often clearer than that of rays that are stored dry. However, further study of both aspects is required.

The purpose of this paper is to describe a new method of estimating both the freshwater and ocean age of chinook salmon spawners from examination of the dorsal fin ray growth pattern. The use of known-age (coded-wire tagged) fish provided evidence of the validity of this method of age determination.

Materials and Methods

Source of Samples

Scale and fin ray samples from chinook salmon spawners were taken from fish of known and unknown ages. The known-age samples were obtained from returning spawners that had

TABLE 1. Estimates of the percent age composition of chinook salmon spawners from various river systems (in 1983), derived from examination of both dorsal fin rays and scales and from only scales.

Location	Method	1.1	1.2	1.3	1.4	1.5	1.6	0.1	0.2	0.3	0.4	0.5	0.6	*N*
Quesnel	Scale	8.2	65.3	26.5	0	0	0	0	0	0	0	0	0	49
	Fin/scale	4.1	4.1	83.7	8.1	0	0	0	0	0	0	0	0	49
Nazko	Scale	23.8	76.2	0	0	0	0	0	0	0	0	0	0	21
	Fin/scale	0	28.6	71.4	0	0	0	0	0	0	0	0	0	21
Blackwater	Scale	18.7	81.3	0	0	0	0	0	0	0	0	0	0	32
	Fin/scale	0	15.6	84.4	0	0	0	0	0	0	0	0	0	32
Chilko	Scale	3.0	65.0	32.0	0	0	0	0	0	0	0	0	0	40
	Fin/scale	0	5.0	80.0	15.0	0	0	0	0	0	0	0	0	40
Bowron	Scale	9.7	77.4	9.7	3.2	0	0	0	0	0	0	0	0	31
	Fin/scale	0	22.6	74.2	3.2	0	0	0	0	0	0	0	0	31
Loon Lake	Scale	73.3	26.7	0	0	0	0	0	0	0	0	0	0	15
and Creek	Fin/scale	6.7	73.3	20.0	0	0	0	0	0	0	0	0	0	15
Stuart	Scale	4.9	90.2	4.9	0	0	0	0	0	0	0	0	0	41
	Fin/scale	0	9.7	87.8	2.4	0	0	0	0	0	0	0	0	41
Nitinat	Scale	0	0	0	0	0	0	0	46.7	33.3	20.0	0	0	15
	Fin/scale	0	0	0	0	0	0	0	46.7	33.3	20.0	0	0	15
Big	Scale	0	0	0	0	0	0	0	6.9	86.2	6.9	0	0	29
Qualicum	Fin/scale	0	0	0	0	0	0	0	3.4	82.7	14.0	0	0	29
Robertson	Scale	0	0	0	0	0	0	0	12.2	69.4	18.4	0	0	49
Creek	Fin/scale	0	0	0	0	0	0	0	14.3	67.3	16.3	2.0	0	49
Conuma	Scale	0	0	0	0	0	0	0	15.8	84.2	0	0	0	19
	Fin/scale	0	0	0	0	0	0	0	5.2	94.8	0	0	0	19
Quinsam	Sale	0	0	0	0	0	0	0	7.9	21.0	28.9	42.1	0	38
	Fin/scale	0	0	0	0	0	0	0	7.9	21.0	15.8	50.0	5.3	38
Terrace	Scale	3.3	0	0	83.3	13.3	0	0	0	0	0	0	0	30
	Fin/scale	0	3.3	0	80.0	16.7	0	0	0	0	0	0	0	30
Kitimat	Scale	0	0	30.4	65.2	4.3	0	0	0	0	0	0	0	23
	Fin/scale	0	0	0	73.9	26.1	0	0	0	0	0	0	0	23
Bella	Scale	0	26.7	73.3	0	0	0	0	0	0	0	0	0	15
Coola	Fin/scale	0	0	26.7	60.0	6.7	6.7	0	0	0	0	0	0	15
Tenderfoot	Scale	3.2	35.5	58.1	3.2	0	0	0	0	0	0	0	0	31
	Fin/scale	3.2	22.6	61.3	12.9	0	0	0	0	0	0	0	0	31

been marked by removing the adipose fin and tagged with coded-wire nose tags (CWT) prior to their release, as juveniles, from five Vancouver Island hatcheries (see Table 1). These samples were all of the age 0. life history type.[1] Additional samples from spawners of unknown age were taken from several stocks along the coast and in the interior of British Columbia (see Table 2). These comprised a mixture of age 0. and age 1. life history types. Scales and fin rays from fish of each sample were examined and ages were determined.

Preparation of Scales and Fin Rays

In the laboratory, acetate impressions were made of the scales using a hydraulic scale press similar to that described by Clutter and Whitesel (1956). The scale impressions were examined at 40−100× magnification using a microprojector.

Each fin was trimmed of its first ray and of any excess flesh and then air-dried. The first ray was removed because we found its growth pattern to be indefinable. Only ray numbers 2−7 were used from each fish for age determination. Sections from more than one ray were useful because growth pattern variations could be more readily interpreted on some rays than on others. If the fin rays were from a very large fish, they were divided into two sections, each made up of two or three rays to facilitate handling and sectioning. A thin layer of epoxy was applied to the basal area of the rays. Sections (usually five or six) approximately 1.0 mm thick were cut from the fin using a Bronwill bone-sectioning machine as described by Chilton and Beamish (1982). Sections were fastened onto glass slides with a liquid cover slip and examined at 40× magnification by transmitted light using a compound microscope.

Description of Fin Ray Sections

When sections are viewed with a compound microscope and transmitted light, wide opaque zones alternating with narrow translucent zones are noted (Fig. 1). An opaque zone represents material deposited during the summer or period of rapid growth, and a translucent zone represents material deposited in the winter or period of slow growth of the fish. These zones therefore resemble the growth pattern found on the scales.

The identification of the freshwater annulus on the fin ray section was made subjectively from the size, the unique tear-shape of the annulus, and the distance to the first marine annulus (Fig. 2). However, when a freshwater check is fairly close to the centre (indicating an early transition to the marine environment), it might be confused with a freshwater annulus (Fig. 3A and 3B); nevertheless checks usually differ in shape from an annulus.

The method of annulus formation in fin rays is currently being investigated. We believe that fin rays grow by the addi-

[1]The European system of age designation (Koo 1962) is used here. The first digit indicates the number of annuli formed in freshwater and the second digit the number of annuli formed in the ocean. For example, a 1.2 fish returns in its 4th yr after spending 1 yr in freshwater and 2+ yr in the ocean.

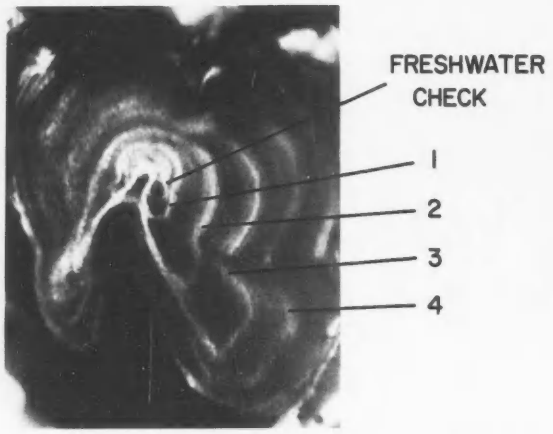

FIG. 1. Thin cross-section from a chinook salmon dorsal fin ray, aged as 0.4 yr, showing a freshwater check and the alternating wide opaque summer growth zones and the narrow translucent winter zones.

tion of acellular bone to the exterior of the ray (R. J. Beamish, pers. comm.). Therefore, in order to retain the evidence of the 1st yr's growth, it is necessary to remove the fins as close as possible to the body and to include the point of articulation. The first section must be cut extremely close to the base of the fin. Often this section will reveal only the freshwater zone and occasionally the first marine annulus. The other marine annuli are barely visible (Fig. 2). The latter will be visible on sections cut distally to the point of articulation (further along the ray) where the freshwater zone gradually disappears (Fig. 4).

Test to Compare Age Estimates Derived from Dorsal Fin Rays and Scales with Estimates from Scales Only

The age composition of chinook spawners in samples from 16 locations was estimated by examination of both the fin ray and the scales from the same fish (Table 1). The scales were examined to try to determine the freshwater residence indicated from the fin rays. However, among fish of both known and unknown age, the freshwater age could not always be determined from their scales because of a fairly high incidence of regenerated scales among the samples. Regenerated scales (Fig. 5) are unreadable because varying amounts of the central portions of the scales are missing. Also, many of the scales among the samples were resorbed (Fig. 6A).

Test for Precision[2] in Determination of Freshwater Age from Scales and from Fin Rays

For this test, fin ray samples from four or five fish of known and unknown ages from each of the 16 locations given in Table 2 were assembled, randomly mixed, and assigned identification numbers. This number only was available to the reader. Three weeks later, ages were again determined under the same conditions by the same reader. An estimate of the precision between scales and fin rays in discriminating a freshwater annulus from an ocean annulus was obtained.

[2]Precision refers to the degree of reproducibility and thus measures the variability between readers or between readings. Validity (accuracy) is a measure of closeness to the true value and thus measures the departure from the "true age" (Chilton and Beamish 1982; Prince and Pulos 1983).

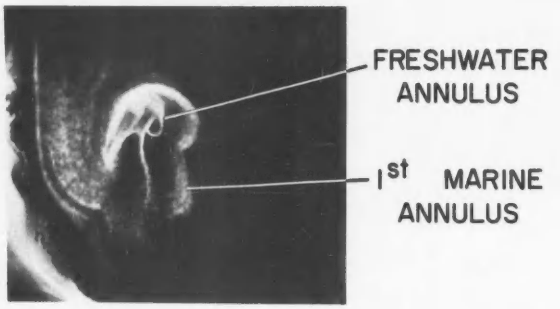

FIG. 2. Thin cross-section from a chinook salmon dorsal fin ray showing a clear tear-shaped freshwater annulus and the first marine annulus. Note that the rest of the ray is opaque. The other marine annuli are barely visible.

Test for Precision in Designation of Total Age (Freshwater plus Ocean Age) Determined from Fin Rays and Total Age from Scales

The same samples used in the test for precision in identification of freshwater age (see previous section) were used in this test.

Test for Validity of Ages Derived from Fin Rays

This test involved examination of fin ray samples from chinook salmon of known age (recovered CWT fish) from five British Columbia hatcheries (see Table 4). Only those samples where estimates of age from fin rays differed from the known age of the fish were examined (see Table 5). A further test of validity in the estimation of both freshwater and ocean age from fins of known-age chinook salmon was carried out (see Table 6). In this test the locations of origin were not known to the reader. Known-age fin ray samples (CWT fish) were used in this test and were aged on two occasions. Although the sample size was small, it does provide some estimate of the accuracy of the method.

Analysis of Data

Scale and fin ray age data for fish of known and unknown ages were summarized by freshwater and ocean age groups. Percentage composition of fish in the samples was calculated. The results of some tests were compared statistically using the Student *t*-test or chi square test (Langley 1979).

Results

Comparison of Age Estimates Derived from both Dorsal Fin Rays and Scales with Estimates from Scales Only

The freshwater ages of the fish in the samples derived by the two methods did not differ (Table 1). This was expected, as the designation of freshwater age was made mainly from examination of scales in both cases. However, the ocean age of the fish derived by the two methods did differ (Table 1). Among samples from all but one location (Nitinat), the scale plus fin ray method estimated the fish to be of an older ocean age than did the scale only method (i.e. often more ocean annuli were

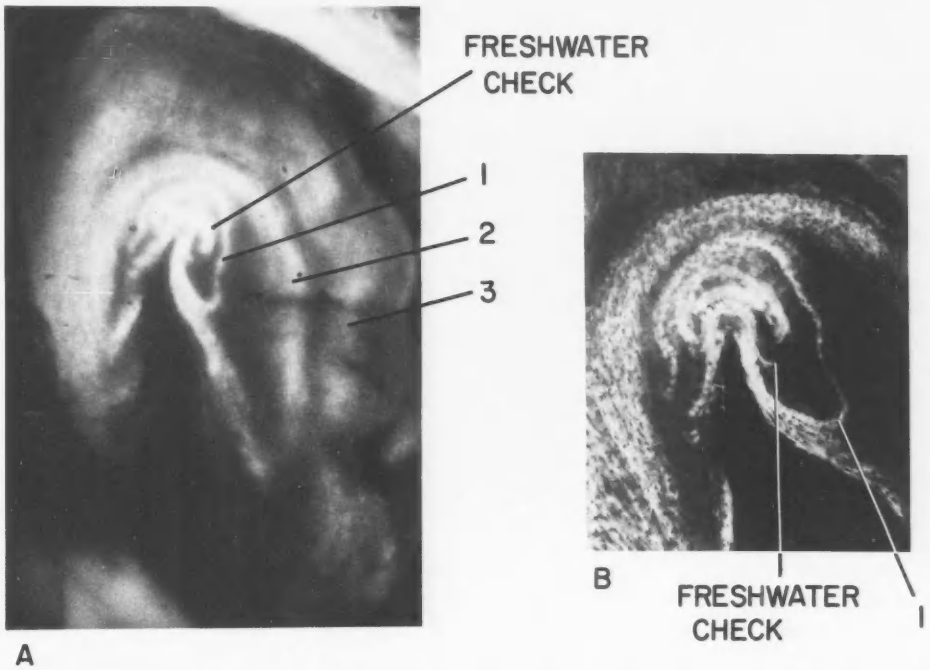

FIG. 3. (A) Thin cross-section from a chinook salmon dorsal fin ray indicating a freshwater check fairly close to the centre and three marine annuli. (B) Enlargement of the freshwater check and the first marine annulus.

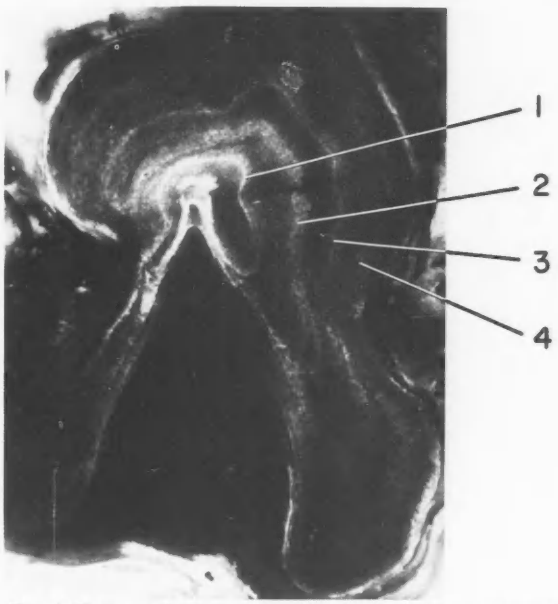

FIG. 4. Two cuts further up the chinook dorsal fin ray shown in Fig. 2. The freshwater annulus is missing but the marine annuli are now visible. It was aged as 1.4 yr. The freshwater residence was aged on the section seen in Fig. 2.

detected on the fin rays than on the scales). In the case of the Nitinat sample the ocean age composition was the same using both methods. The overall (samples from all 16 locations combined) mean ocean age of fish in the samples derived by the two methods was statistically different ($\bar{\bar{X}}$ ocean age from fin rays was 3.17 yr and from scales was 2.64 yr; $t = 5.048$, df = 15, $P < 0.005$). The overall ocean age composition of the fish in the samples derived by the two methods was compared using chi square. Results of the test indicated that the age compositions differed significantly ($\chi^2 = 142.28$, df = 5, $P < 0.01$). Hence, the data indicate that scales by themselves in many cases underestimated the representation of older fish in the samples. Examination of the condition of the scales indicates that the underageing of the fish was due to scale resorption and not to errors in interpreting the ocean annulus (see example given in Fig. 6A).

TABLE 2. Agreement in assignment of freshwater age as determined from scales and fin rays and mixed unknown and known-age chinook salmon.

Freshwater age as aged from scales	Agreed, first reading		Agreed, second reading	
	No.	%	No.	%
0.	16/17	94.1	14/17	82.3
1.	32/35	91.4	32/35	91.4
Total	48/52	92.3	46/52	88.5

Precision in Determination of Freshwater Age from Scales and from Fin Rays

In the first reading of the fin rays the agreement in freshwater ages between fin rays and scales was high (92.3 ± 7.2% at the 95% confidence level) (Davies and Goldsmith 1980) (Table 2).

TABLE 3. Percent age composition of chinook salmon of mixed known and unknown age estimated from their scales and fin rays. Fin rays were read on two separate occasions.

	0.2	0.3	0.4	1.1	1.2	1.3	1.4	1.5	Total %	*N*
Scales	7.7	15.4	9.6	5.8	38.4	17.3	5.8	0.0	100.0	69
Fin rays										
First reading	5.8	17.3	13.5	0.0	13.5	32.6	15.4	1.9	100.0	69
Second reading	5.8	13.5	13.5	0.0	11.5	38.4	15.4	1.9	100.0	69

FIG. 5. Regenerated scale. Note that a large portion of the centre is missing.

Agreement for fish classified as age 0. and age 1. was 94.1 and 91.4%, respectively. Hence, agreement in assignment of each life history type was about equal ($\chi^2 = 0.16$, df = 2, $P > 0.1$). On the second reading of the same fins, agreement between scales and fin rays was again high (88.5 ± 8.7% at the 95% confidence level). Agreement for fish classified as age 0. and age 1. was 82.3 and 91.4%, respectively. In this case, the agreement was the same as the first reading in assignment of age 1. fish, but less than the first reading for those classified as age 0. fish.

Precision in Designation of Total Age (Freshwater plus Ocean Age) Determined from Fin Rays and Total Age from Scales

The age composition derived from fin rays on the two occasions (Table 3) could not be demonstrated to be significantly different ($\chi^2 = 0.8$, df = 7, $P > 0.1$). In both readings, age 1.3 fish represented most of the fish in the sample. Comparison of both age compositions derived from the fins and from the scales indicated a significantly high proportion of older fish in the former than in the latter (χ^2 values for first and second readings of fin rays with scales were 19.46, df = 7, $P < 0.01$ and 24.08, df = 7, $P < 0.01$, respectively). Hence, the ages of many of the older fish were not detected from their scales because of resorption.

TABLE 4. Number and percentage of agreements between ages determined from dorsal fin rays and known age of spawners as derived from coded wire tags (CWT).

	Agreed		Disagreed		
Location	No.	%	No.	%	Total
Quinsam	31	81.5	7	18.5	38
Nitinat	15	93.7	1	6.3	16
Conuma	27	96.4	1	3.6	28
Robertson Creek	51	92.7	4	7.3	55
Big Qualicum River	39	88.6	5	11.4	44
Average		90.6		9.4	

Validity of Ages Derived from Fin Rays

In general, the agreement was high between ages determined from fin rays and known age of the fish (Table 4). It averaged 90.6% and ranged from a low of 81.5% for Quinsam fish to a high of 96.4% for Conuma fish. These results indicate that it is possible to age chinook spawners, from examination of the dorsal fin, with a high degree of accuracy. Table 5 examines only those samples where estimates of age from fin rays differed from the known age of the fish. The reader made errors of both overageing and underageing. However, consistent bias one way or the other could not be demonstrated statistically.

In the further test of validity, where estimation of both freshwater and ocean age of known age chinook salmon was made (Table 6), the results indicated that the age composition from fins read on two occasions did not differ significantly from the true age composition of fish in the sample (i.e. first reading $\chi^2 = 1.28$, df = 3, $P > 0.1$; second reading $\chi^2 = 2.80$, df = 4, $P > 0.1$). With the known-age 0.2 fish, some difficulty was encountered. In the first reading, 75% were correctly designated and 25% were incorrectly classified as age 0.3 fish. In the second reading, only 50% of age 0.2 fish were correctly classified and the remaining 50% were misclassified equally as age 0.3 and 1.2 fish. In both readings of the known-age 0.4 fish, 83.3% were correctly classified, with the remaining 16.7% misclassified as either age 1.2 or 1.3 fish.

Discussion

Fin rays have been used to estimate the age of sockeye and chum salmon spawners (Bilton and Jenkinson 1969). However, the former fish may spend 1 or more yr in freshwater before migrating to the sea, and identification of a freshwater annulus on the fin ray has not been possible. To obtain the total age of sockeye salmon, a combination of scales (to estimate freshwater age) and pectoral fin rays (to estimate the ocean age) was used. For chum salmon the age was determined by examination of the pectoral fin rays only. For chinook salmon, which spend

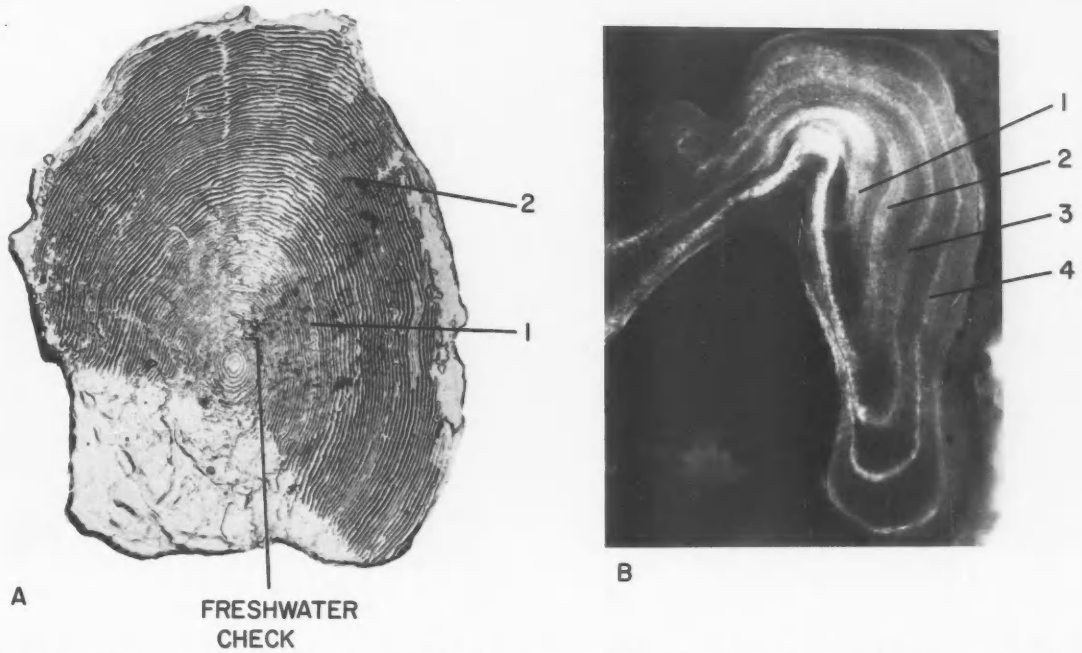

FIG. 6. (A) Resorbed scale from a mature known-age (0.4 yr) chinook salmon indicating two marine annuli and a freshwater check. (B) Thin cross-section from the dorsal fin ray of the same coded-wire tagged fish showing four marine annuli, thus establishing the true age of the fish. The freshwater check is missing from this fin ray section. It was visible on a section cut closer to the base of the fin; however, the marine annuli were opaque on this section.

TABLE 5. Differences in estimated ages between fish aged from fin rays and from the coded-wire tag (CWT).

CWT age	Fin age	Number of times age of Fin > CWT	Number of times age of Fin < CWT	Difference in age (yr) of fin from CWT
0.4	0.5	2		+1
0.4	0.3		2	−1
0.3	0.4	7		+1
0.3	0.2		6	−1
0.2	0.3	1		+1
Total		10[a]	8[a]	

[a]50% probability test indicates probability >5%; hence, the difference is not proven statistically.

TABLE 6. Agreement between readings on two occasions of freshwater and ocean age from fin rays and known age of chinook salmon.

True age	No. of fins	Assigned age from fin ray 0.2	0.3	0.4	1.2	1.3
		First reading				
0.2	4	75.0	25.0			
0.3	8		100.0			
0.4	6			83.3	16.7	
Total	18					
		Second reading				
0.2	4	50.0	25.0		25.0	
0.3	8		100.0			
0.4	6			83.3		16.7
Total	18					

from several months to a year or more in freshwater before migrating to sea, accurate identification of the freshwater age on any ageing structure has been difficult because criteria are lacking upon which an interpretation can be based with confidence. However, in this study, in contrast with the earlier study on sockeye (Bilton and Jenkinson 1969), improved techniques in sectioning, removal of the fins from the fish, and in preservation suggest that it is now possible to distinguish freshwater annuli on the dorsal fin rays of chinook salmon.

The method of estimating age of chinook salmon spawners from a combination of dorsal fin rays and scales is superior to the method using only scales for several reasons. First, the fin ray method generally provided the same estimates of freshwater age of the spawners as did the scale method. The precision between the two methods in estimating the freshwater age of the fish was high. Validation or accuracy in estimating the freshwater age of age 0. type fish of known age was also high. It should be pointed out, however, that this test had several shortcomings. For example, the reader knew the locations from which the samples had been obtained. The reader was also aware that hatcheries almost always release juvenile chinook early in their 1st yr of life as age 0. Hence, the chance of there being a substantial representation of fish that had spent a full year in freshwater (age 1.) was quite remote. The reader could conceivably have been biased by this fact. Further testing of the fin ray method is required using fin rays from known-age chinook salmon that have spent 1 yr (age 1.) in freshwater before going to sea.

Second, tests of the validity of the fin ray method suggest that ocean age can be estimated with a high degree of accuracy (Fig. 6B), indicating that the method should generally provide reliable estimates of the age composition of populations of chinook spawners.

Third, comparison of age estimates derived from examination of both dorsal fin rays and scales, with scales only, indicates that the *scale method by itself underestimates the age composition of the sample*. This is because scales underestimated the ocean age by 1 or more yr. Underestimation of ocean age using scales is related to the resorption of the scales as the salmon mature; this results in a loss of one or more ocean annuli over time.

Because errors in ageing can lead to faulty estimates of production from various brood years and can therefore result in improper management of the stocks, managers should be encouraged to use a combination of scales and fin rays to estimate the total age of spawners.

Acknowledgments

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Diel Distribution and Activity of American Eels (*Anguilla rostrata*) in a Cave-Spring

Gene S. Helfman

Department of Zoology and Institute of Ecology, University of Georgia, Athens, GA 30602, USA

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American eels (*Anguilla rostrata*) in a Florida cave-spring (total population ≅ 142 eels, density ≅ 0.03 eels/m^2) showed distinctive day versus night differences in distribution and activity. Larger eels occurred deeper at all times. Eels were diurnally quiescent, nocturnally active, and changed over between behavior modes roughly at dusk and dawn, although considerable variability existed in timing of changeover. Changeover at dusk involved movement from deeper regions of low food to shallower regions of high food concentration, with smaller eels more likely to migrate. Changing light levels apparently determined onset and cessation of activity, with an inhibitory threshold of 10–100 lx influencing movement between deep and shallow regions. An endogenous rhythm and hunger may have also affected activity patterns.

On a constaté au sein d'une population d'anguilles d'Amérique (*Anguilla rostrata*) observées dans une source de caverne en Floride (population totale ≅ 142 anguilles, densité ≅ 0,03 anguille/m^2) une différence très marquée dans leur répartition et leur activité le jour et la nuit. Les plus grosses anguilles se tenaient constamment en eau profonde. Les anguilles flottaient passivement le jour et étaient actives la nuit, passant généralement d'un comportement à l'autre au crépuscule et à l'aube bien que le moment exact du passage variait considérablement. Le changement de comportement au crépuscule impliquait une remontée des régions profondes offrant peu de nourriture à des régions de plus faible profondeur où la nourriture était abondante; les petites anguilles étaient plus aptes à la migration verticale quotidienne. L'intensité de pénétration de la lumière solaire détermine apparemment le début et la fin de leur période d'activité, le seuil inhibitif de 10–100 lx déclenchant leur déplacement de la zone profonde à la zone superficielle. Un rythme endogène et la faim peuvent aussi avoir eu un effet sur le schéma de leur activité.

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Field investigations of the daily activity patterns of fishes indicate that most species are active by day or by night, with some species active primarily during crepuscular periods of dusk and dawn (e.g. Hobson 1975; Helfman 1978, 1986a). Fishes generally initiate and cease activity at relatively fixed times, although timing varies with latitude and habitat type (Helfman 1981; Hobson et al. 1981; Hobson 1986). Our knowledge of the diel activity patterns of diurnal fishes is extensive. However, field information on nocturnal species is limited to tropical marine fishes (e.g. Dale 1975; Ogden and Ehrlich 1977; Gladfelter 1979; Gladfelter and Johnson 1983; but see Ryder 1977). Recent technological developments (light amplification devices, low-light video-cameras) have apparently not been used to investigate activity cycles of nocturnal fishes (Helfman 1983).

American and European eels, *Anguilla rostrata* and *A. anguilla*, are among the best-studied fish species in the world, yet little is known about the behavior of either species (Tesch 1977). Many fisheries for these eels depend heavily on successful interception of animals during their daily foraging movements. However, direct diver or biotelemetric observation of the timing of such movements and the factors that influence them are limited (e.g. Helfman et al. 1983; LaBar and Facey 1983). Beyond general, and sometimes debated, statements that anguillid eels are nocturnal foragers (reviewed in Ryan 1984), little quantitative data exist.

The objectives of the present study were to capitalize on a locale with year-round eel activity and clear water and use diving and underwater videocamera observations to determine (1) daytime and nighttime distribution patterns, (2) diel patterns and times and light levels at onset and cessation of activity, and (3) population size, density, and biomass in a population of free-living American eels. Population data were collected as measures of diel distribution and also as indicators of whether generalized day/night patterns characterized a significant fraction of the stock. In addition, density and biomass of potential invertebrate prey were determined because of the influence prey distribution might have on eel distribution.

Materials and Methods

Study Site

Vortex Blue Spring (Holmes County, Florida, 30°46′14″N, 85°56′55″W) is a third-magnitude, artesian spring with an average flow rate of 200 L/s, 19–23°C temperature range, and 5.3 mg/L dissolved oxygen (Rosenau et al. 1977). Horizontal underwater visibility ranges between 10 and 30 m. Water in the spring flows through limestone rock and leaves the basin via a 400-m run, eventually entering the Choctawhatchee River approximately 50 km from the river mouth.

Eels were observed in three regions of the Vortex cave-spring system: the basin, cavern, and tunnel (Fig. 1). The basin

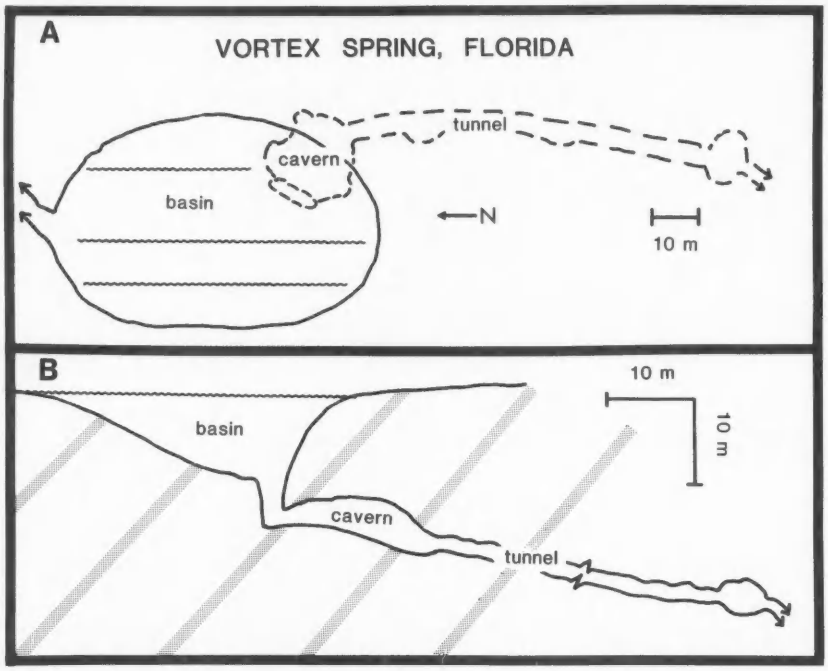


FIG. 1. Vortex cave-spring system, showing relationships and depths of basin (region immediately under air–water interface), cavern (subterranean region illuminated by daylight), and tunnel (subterranean region receiving little or no daylight). (A) Top view; (B) lateral view; note different scales. Arrows to the north indicate spring run and to the south indicate continuation of tunnel.

is topographically complex, covered by vegetation, sand, and silt, with average water depths of 5–10 m. The basin slopes to a depth of 11 m, where a 7 × 2 m vertical chimney leads down to the cavern mouth at 15 m. The cavern floor is covered with sand, silt, and debris (*Goniobasis* snail shells and *Spirogyra* algae). The cavern slopes to 17 m, leading to the 1 m high × 4 m wide tunnel mouth. The tunnel has a sand-covered floor, limestone walls, and is 1–2 m high × 3–6 m wide, with several side rooms. Visible light penetrates to approximately 60 m past the tunnel mouth. The owners of the spring have blocked the tunnel with a wide-mesh grating at 34 m depth to prevent deeper penetration by divers.

Distribution

Patterns of distribution were determined by counting eels along a line transect (Helfman 1983). Counts were performed by two divers swimming together and counting their respective sides of the transect. Underwater flashlights were used at night and in darker portions of the cavern and tunnel, although care was taken to shine lights only a few metres ahead to avoid frightening uncounted eels. Eels seldom fled from divers and showed little reaction to light unless it was shone directly in their eyes.

The transect sampled the entire cavern (176 m^2) and tunnel down to 28 m (600 m^2), but subsampled the basin. In the basin, eels were counted within 3 m (estimated) of either side of a 40-m-long rope that ran from the shoreline to the cavern mouth (counts were conducted between ropes 6 m apart in January 1985). All eels were counted on either side of a permanent guide line that runs down the middle of the cavern and tunnel. Side pockets, crevices, and ledges were also searched. Approximate size and locale were recorded for each eel sighted. Sizes were estimated by comparison with a ruler held as close as possible to the eel. Comparison of actual and estimated sizes indicated errors of ±2–3 cm.

Counting was conducted on 10 day and 13 night occasions during July 1982, March 1983, July 1983, and January 1985. Counts occurred during all major time blocks of the day and night. Decompression limits made it impractical to perform sequential, replicated counts. Because eels are fossorial, counts are likely underestimates of actual numbers.

Day versus night differences in density, biomass, and size were compared using survey data from counts during a 24-h period (e.g. day 1/night 1, night 1/day 2, etc.). Density estimates for the entire basin (4200 m^2) were extrapolated from the number of eels seen on the transect in the southern section (240 m^2). Estimates for the entire basin were adjusted by determining relative frequencies in the south, middle, and north sections of the basin on four nights in January 1985. To determine biomass, the proportion of the population in each 5-cm size class (based on the length–frequency distribution for July 1983, 400 eels, six counts) was multiplied by the average number of eels occurring in each region by day and night. The resulting values were multiplied by the average weight of each 5-cm size class, determined from a curvilinear regression of weight on length (weight = 1/ln (length) (−0.00944) + 0.0640, $r = -0.872$, $P < 0.001$, df = 37 Vortex eels). These weight/size class values were then summed and divided by the area of the region to produce biomass per square metre (see Bozeman et al. 1985). Diel

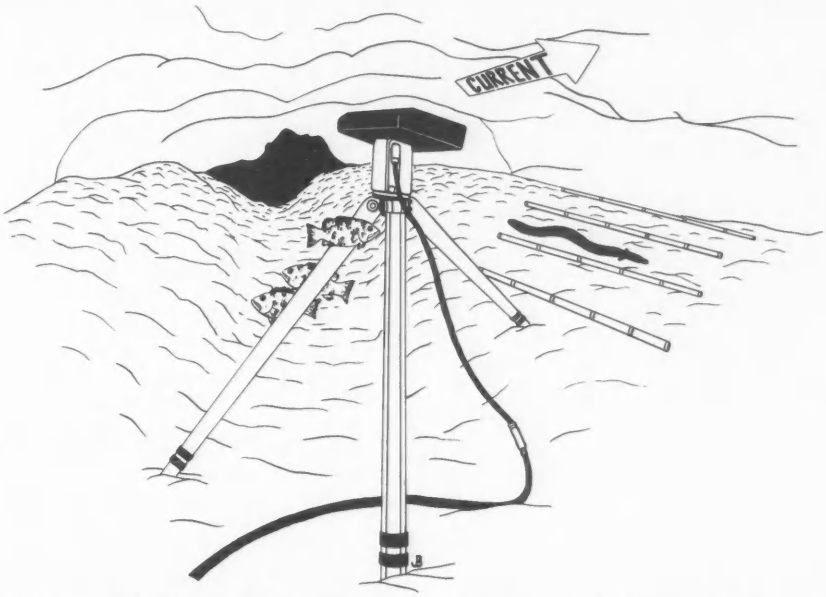


FIG. 2. Monitoring American eel activity at tunnel mouth, Vortex Spring. Videocamera sits in approximate center of tunnel mouth, pointing at west wall of tunnel. Metre-sticks in field of view aid in determination of size and speed of passing eels. View of camera is from tunnel mouth looking down tunnel.

differences in eel size in different regions were compared with the General Linear Methods Procedure (ANOVA), followed by Duncan's multiple range test (Helwig and Council 1979).

Population size was calculated in two ways. Mean values for all surveys (both day and night) were combined to provide overall estimates of population parameters. As a second estimate of abundance, population size was calculated from the six nighttime surveys when counting was conducted in all three regions of the spring.

Activity

Activity patterns were analysed from videotapes and diver observations. Beginning March 1983, 105 h of observation were made with an underwater television (Video Sciences Inc., Chatsworth, CA). The videocamera was a monochrome Panasonic unit equipped with an 8-mm F1.5 lens, low-light Newvicon tube, and two video lights (6 W diffuse illumination), and connected by a 75-m cable to an RCA videocassette recorder and a monitor that displayed time and date. The camera was placed in the center of the tunnel mouth, facing four metre-sticks laid parallel to the direction of movement of most eels (Fig. 2). Videotapes were analyzed for time and speed of movement of eels exiting and entering the tunnel. Eel size was estimated to the nearest 5 cm by comparison with the metre-stick closest to the eel in question.

To determine periods of onset and cessation of activity ("changeover"), we set the camera at the tunnel mouth and filmed continuously, with some interruptions, for 3 d in March 1983 and 6 d in July 1983. Tapes were analyzed for directional, one-way movements past the camera as a function of time of day. Net movement out or in was taken as evidence of initiation and cessation of activity, respectively; periods of no net movement (either outward moves = inward moves, or no movement at all) were considered transitional or inactive periods. A nondirectional move involved one animal passing back and forth across the field of view of the videocamera during <2 min. A back-and-forth pass in front of the camera was counted as one move.

To determine time of changeover more precisely, directional movement data were analyzed over 15-min intervals from 2 h before to 2 h after sunset and sunrise. Changeover was also characterized by determining the time when most eels had completed movements out of or into the tunnel. Using data from the three sunset and five sunrise occasions with complete videotape records, cumulative hourly percentages were calculated for all eels leaving the tunnel between 4 h before and 2 h after sunset and returning between 6 h before and 2 h after sunrise. Cumulative percentages were then regressed against time and the resulting equations used to determine quartiles of activity, i.e. time (x-axis) when 25, 50, and 75% (y-axis) of eels had passed the camera.

As size differences in changeover time are common among fishes (Helfman 1986a), the possibility of a relationship between time of emergence from or return to the tunnel mouth and eel length was tested by regressing eel length on time, using the video record from 11–12 July 1983 (the 24-h period when the most eels were seen).

The videocamera viewed approximately one half of the tunnel mouth and provided weak illumination. Eels avoiding the lights could have exited or entered the tunnel behind the camera, thereby biasing changeover results. To assess possible camera-induced bias, numbers of eels exiting versus entering were compared for a 48-h continuous recording period in July 1983.

The size and complexity of the cavern mouth made camera

observations there impractical. Diving observations of twilight changeover were therefore made at both the cavern and tunnel mouths, the latter to check the accuracy of the camera-derived data. Data were obtained during 23 dusk and 16 dawn dives. Divers used red-filtered flashlights or a light amplification device in a Plexiglas underwater housing (Astrophysics Inc., Nutley, NJ), with supplemental illumination (Cyalume Lightsticks, American Cyanamid, Bound Brook, NJ). General activity and time were recorded, with particular attention to number of eels leaving and entering the tunnel or cavern. Depth and time constraints limited observations to the 2 h preceding and 2 h following sunset and sunrise.

Light and Time

Maximum available light (luminance) was measured with a Gossen Lunapro light meter in an Ikelite underwater housing, calibrated with a Gamma model 3000 SR recording spectroradiometer (see McFarland et al. 1979; Helfman 1981). Downwelling light was measured in the cavern mouth (seven dusks, five dawns), whereas horizontal or angular spacelight were measured in the cavern and tunnel (three daytime occasions). Light measurements have been converted to approximate lux, using conversion factors provided by the manufacturer. Times of activities were recorded to the nearest minute, Central Standard Time. Times of sunset (SS) and sunrise (SR) were calculated from the *American Ephemeris and Nautical Almanac* (Anonymous 1974). Times are given relative to sunset and sunrise: minus (−) values are before and plus (+) values after sunset and sunrise.

Benthic Invertebrate Samples

In January 1985, 10 benthic invertebrate samples were obtained during daylight by divers from each of the tunnel, cavern, and basin using a 75-mm-diameter corer pushed 30 mm into the sediments (Downing 1984). Sampling sites were determined by haphazardly thrown quadrats; we sampled the entire basin and cavern, but only the first 10 m inside the tunnel mouth. Samples were preserved in 10% formalin, stained with phloxin-B, washed, and filtered through a 0.15-mm sieve. All organisms were identified to the lowest possible taxon and counted. Biomass was determined after drying samples, including soft parts of snails, in an oven at 50°C for 24 h. Dried samples were weighed to the nearest 0.1 mg; results are expressed as milligrams per square centimetre of bottom. Snail densities were also sampled at nine locales in each region by haphazardly placing a 480-cm^2 square wire frame against rocky substrates and picking all snails within the frame. Snail counts in the various regions were compared by the nonparametric Kruskal–Wallis Multiple Comparisons Test (Hollander and Wolfe 1973).

Results

Diel Distribution and Abundance

In the basin, we saw one eel during six daytime counts and 64 eels during 10 nighttime surveys (Table 1). A 10-fold increase in observed biomass at night paralleled the nighttime increase in density. Basin transects at night indicated that eels were more common in the deeper half of the basin, with a mean of 83% (SD = 25%, N = 12 counts) occurring in water ≧3 m, and a mean of 83% (SD = 3%, N = 4 comparisons) of

TABLE 1. Diel patterns of abundance and distribution of American eels in the Vortex cave-spring system based on transect surveys. Most values are means per survey, with standard deviations in parentheses. "Total seen/no. surveys" are absolute values, and "Overall" values are sums of the different regions in the respective column. An asterisk indicates significant differences ($P < 0.05$) in means between day and night based on Student's t-test (abundance) or analysis of variance (size); ns = not significant ($P > 0.05$).

	$\bar{X}$ no. eels/survey		$\bar{X}$ density (eels/m^2)		$\bar{X}$ size (cm)		$\bar{X}$ biomass (g/m^2)		Total seen/no. surveys	
	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
Basin (240-m^2 transect)	0.2 (0.4)	6.4 (4.2)*	0.0008 (0.002)	0.03 (0.02)	43 (—)[a]	34.3 (10.1)	0.5	4.8	1/6	64/10
Cavern (176 m^2)	23.6 (5.2)	22.4 (7.0) ns	0.14 (0.03)	0.13 (0.04)	34.9 (8.6)	40.3 (9.7)*	18.2	20.3	213/9	185/9
Tunnel (600 m^2)	31.1 (18.8)	16.8 (6.8)*	0.05 (0.03)	0.03 (0.01)	42.8 (10.6)	48.3 (13.4)*	12.5	8.0	248/8	134/7
Overall[b] (4976 m^2)	58.2	128.8	0.01	0.03	39.2 (11.4)	42.3 (12.8)*	12 783[c]	28 501[c]	462	383

[a]Only one eel was seen during six daytime surveys in the basin.
[b]Overall abundance, density, and biomass extrapolated to entire basin (4200 m^2), corrected for 20% overestimation of basin abundance due to counts in southern section of basin only.
[c]Represents total biomass (g) in all 4976 m^2 of cave-spring system (tunnel + cavern + basin).

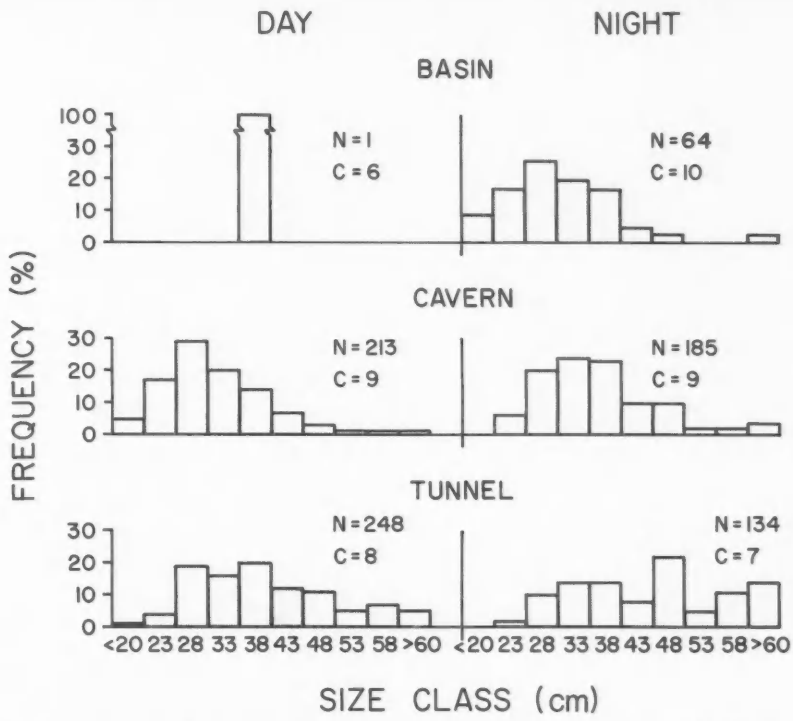


FIG. 3. Day versus night patterns of size–frequency distribution of American eels in different regions of Vortex cave-spring system. Data grouped into 5-cm size classes. Frequencies at each site and each time based on combined samples from all transect surveys, with number of eels seen (N) and number of counts (C) shown for each locale–time combination.

animals in the southern two thirds of the basin (χ^2 of differences between depths or regions, $P < 0.025$, df = 1 in both comparisons). These patterns suggest that nocturnal abundance of eels was greatest closest to the chimney leading to the cavern (see Fig. 1).

The cavern contained the greatest density of eels during both day and night (Table 1). Eel abundance in the cavern did not differ between day and night ($P = 0.68$, t-test, two tails; Table 1). Despite constant density, average size of animals increased on all 11 nights ($P < 0.05$, ANOVA; Fig. 3). Constant density and increased size were reflected in biomass, which rose slightly at night in the cavern.

In the tunnel, eel abundance decreased at night during eight of nine surveys ($P = 0.04$, t-test, one tail), with one-third fewer eels occurring by night than by day (Table 1). Mean eel size was larger at night during all nine comparisons ($P < 0.05$, ANOVA). Biomass appears to have fallen slightly in the tunnel at night. Approximately one-third of all eels in the tunnel during both day and night occurred beyond the extinction point for visible daylight.

Diel differences in eel size among sites were also evident (Fig. 3). Combined size–frequency distributions for all sampling periods indicated diel differences in the distribution of relatively small versus large animals. Small animals had greater relative abundances in the basin and cavern, whereas larger animals were more abundant in the tunnel ($P < 0.01$, t-test, one tail). Relative abundances of small animals decreased at night in both cavern and tunnel regions (mean density per survey: cavern, 0.03 eels/m^2 day, 0.008/m^2 night, $P < 0.01$; tunnel, 0.003/m^2 day, 0.005/m^2 night, $P = 0.06$, t-tests, one tail). Large (>55 cm) eels constituted 12–25% of the tunnel population and only 2–6% of the cavern and basin populations. Relative abundances of large animals in the tunnel increased at night by 13% but remained relatively unchanged in the cavern (4% increase).

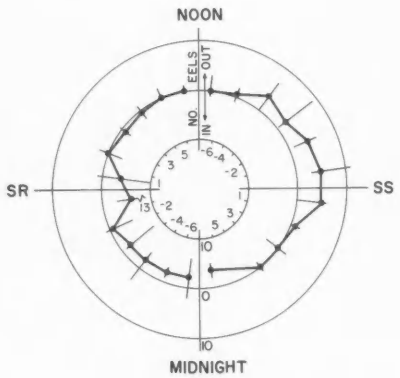
Overall population size, density, and biomass were greater at night than during the day (Table 1), but daytime values are underestimates, as they do not account for hidden animals. These overall values are at best estimates of numbers of exposed (day) and probably active (night) animals. Nighttime-only counts yielded a mean population estimate of 142.3 ± 80.5 eels (±95% confidence interval), or 0.03 eels/m^2 (57 kg/ha), which is intermediate with respect to other American eel stocks (see Bozeman et al. 1985).

Activity Patterns

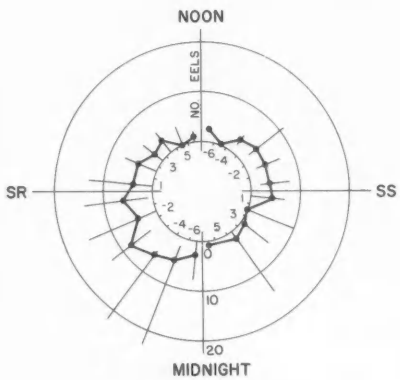
Directional movement

Preliminary observations in Vortex Spring indicated diurnal quiescence and nocturnal foraging: many eels left the tunnel and the cavern in the evening around sunset to forage in shallower water and then returned to the cavern and tunnel the next morning around sunrise.

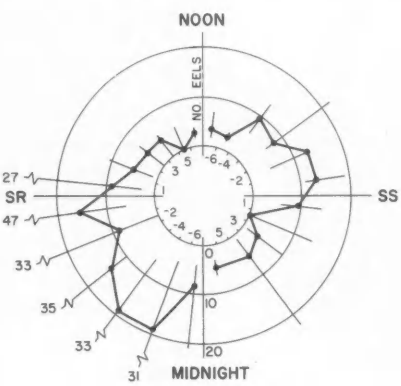
Net movement outward (Fig. 4A) was most evident during late afternoon, beginning about SS − 4 h and peaking during SS ± 1 h. No movement into the tunnel was seen during this period. Net movement fell to zero during the postsunset period with some movement in both directions. Movement into



A. DIRECTIONAL



B. NONDIRECTIONAL



C. TOTAL ACTIVITY

the tunnel characterized the entire predawn period from midnight to sunrise, with a peak at SR ± 1 h. Some eels also moved out of the tunnel during the predawn period, except for the period of maximal inward movement at SR − 1 h. Net movement approached zero by SR + 2 h and remained low during daylight, with small numbers moving both into and out of the tunnel. Analysis over 15-min intervals during the 4-h encompassing sunset or sunrise (Fig. 5) indicated that eels exited the tunnel in the evening over a prolonged period, peaking from SS − 60 min to SS + 30 min. At dawn, numbers of returning animals gradually increased before sunrise, peaking at sunrise and decreasing thereafter.

Sunset and sunrise regressions of percent eels leaving or entering versus time were highly significant ($r > 0.860$, $P \ll 0.01$, df = 15 and 38 for sunset and sunrise, respectively). In the evening, 25% of directionally moving eels had left the tunnel by SS − 1.9 h, 50% by SS − 0.7 h, and 75% by SS + 0.5 h. In the morning, 25% of the directionally moving eels had reentered the tunnel by SR − 3.4 h, 50% by SR − 1.6 h, and 75% by SR + 0.1 h. This analysis confirms the prolonged nature of changeover at both dusk and dawn. No relationship was found between size of eels exiting the tunnel and time in the evening ($r = -0.033$, $P > 0.5$, df = 42). However, a significant negative relationship was found in the morning, indicating a progressive decrease in size of returning animals ($r = -0.392$, $P < 0.001$, df = 68).

Nondirectional activity

Nondirectional activity was also usually low by day and high at night (Fig. 4B). However, some nondirectional activity was evident at all hours except noon ± 2 h. Moderate levels characterized the 4 h before and 1 h after sunset and then decreased during the 2 h after sunset. An increase in nondirectional activity occurred during the midnight to dawn period, when many animals had begun returning to the tunnel (see Fig. 4A). Nondirectional activity peaked 3−4 h before sunrise and then decreased steadily to moderately low, midmorning levels.

Total activity

Total activity was defined as the sum of nondirectional and directional movement (Fig. 4C). Total activity was generally high before sunset, after midnight, and before sunrise, moderate at night before midnight, and lowest throughout the day until late afternoon. All six separate records for different evenings showed a postsunset decline in total activity, particularly in the 1- to 2-h period following sunset. Activity then rose to intermediate levels for the late evening − early morning

FIG. 4. Diel activity of American eels at Vortex tunnel mouth, based on 105 h of nearly continuous video-camera records, March and July 1983. Solid circles represent medians and ranges of numbers of animals passing camera during each one hour period. Negative numbers in inner circle are hours before sunset (SS) or sunrise (SR); other numbers are post-SS and post-SR. All data analyzed relative to time of sunset or sunrise, not to clock time. Breaks in records at noon and midnight represent allowances for differences in daylength and sunset or sunrise times during different months. Camera at tunnel mouth as shown in Fig. 2. (A) Directional activity: values outside "0" circle are medians and ranges of numbers of animals exiting tunnel mouth each hour, whereas values inside "0" circle are animals entering tunnel mouth; (B) nondirectional activity: medians and ranges of numbers passing back and forth before camera, with each value representing a back-and-forth movement by one individual during <2 min; (C) total activity: sum of directional and nondirectional activity in Fig. 4A and 4B.

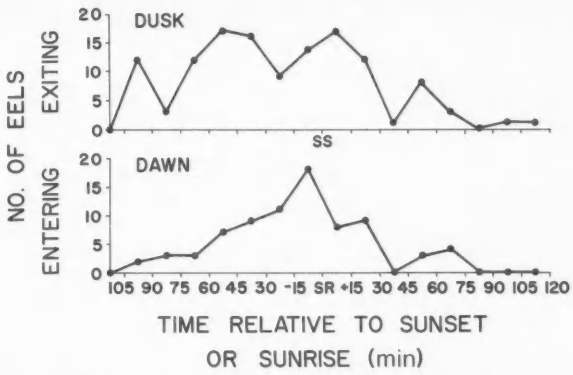


FIG. 5. Directional movement at twilight of American eels, Vortex Spring. Solid circles represent net number of eels exiting or entering tunnel during 15-min intervals. Data are sums of six dusks and six dawns, March and July 1983, based on continuous videotaping. SS = sunset, SR = sunrise.

period before reaching a predawn peak during the 4-h period before sunrise. Activity then decreased rapidly from SR to SR + 2 h. Activity remained low throughout the day and began to increase 3−4 h before sunset, reaching a peak 1−2 h before sunset.

Analysis of 48 h of continuous recording for evidence of camera avoidance showed 94 animals exiting the cave and 98 entering during the first 24-h period and 90 exiting and 79 entering on the second day. Combining the results indicates a net "loss" of seven animals, or 2% deviation from equal numbers exiting and entering. This measure of net movement suggests that the directional activity results are not heavily biased by animals moving in front of the camera at one time and behind it at another. In addition, divers detected neither avoidance of the camera nor differences in behavior of eels in the illuminated area. The relative balance of numbers passing the camera in either direction indicates that individuals may have returned to the same general area to rest each day.

Diving observations

In the evening, a distinct net movement occurred out of both the tunnel and cavern. Of 47 eels passing through the tunnel mouth, 42 (89%) exited. Of 35 eels moving up or down the chimney, 33 (94%) moved up to the basin. Movement out of the tunnel at dusk peaked during the 15-min period prior to sunset and decreased about an hour after sunset (Fig. 6). The rate at which eels exited the cavern in the evening increased at about SS − 15 min, at light levels of about 100−10 lx (Fig. 6). Activity at the cavern mouth was greater after than before sunset ($P < 0.05$, Mann−Whitney U test, two-tail comparisons of mean activity/15-min period, eight periods before and four after sunset).

Activity at the cavern mouth at dusk peaked about 15 min later than at the tunnel entrance. This would be expected if eels moved from the tunnel through the cavern and then up the chimney to the spring basin. Eight of 10 eels followed at dusk as they exited the tunnel mouth stopped en route to forage, rest, or to interact with other fish for 3−43 min; two eels took 1 and 2 min to cross to the cavern mouth, the latter animal continuing up the chimney to the basin. This behavior could account for the later peak time suggested for animals exiting the cavern.

Net directional movement in the morning also occurred in the expected direction. At dawn, 97% of 33 eels moved down the tunnel and 85% of 26 eels entered the cavern. Eels returned to the cavern from the basin prior to sunrise and activity decreased by SR + 15 min. Light levels during the decrease in activity were similarly between 10 and 100 lx. Activity at the cavern mouth was greater before than after sunrise ($P < 0.005$, Mann−Whitney U test, four periods before and eight after sunrise). A gradual increase in numbers of animals entering the tunnel during the hour before sunrise was followed by a decrease over the next hour (Fig. 6).

Illumination

Downwelling illumination at the cavern mouth decreased from daytime maximum values of approximately 20 000 to 75 lx at sunset (Fig. 6; midday light levels 0.3 m below the surface in the basin ranged from 60 000 to 150 000 lx; sunset levels were approximately 200 lx). The 0.001-lx limit of the light meter was reached at approximately SS + 45 min. Dawn light conditions were basically a mirror image of dusk levels. Daytime illumination at the tunnel mouth did not exceed 10 lx. Measurable light (>0.001 lx) did not occur >11 m into the tunnel. Assuming that light in the tunnel mouth underwent reduction in parallel with the pattern at the cavern mouth, light in the tunnel would have fallen to 0.001 lx approximately 20 min before sunset, or 65 min earlier than at the cavern mouth (Fig. 6).

Distribution of benthic prey

The invertebrate fauna in the sediments of Vortex Spring was relatively depauperate and was dominated numerically by aeolosomatid oligochaetes (68%) and gammaridean amphipods (*Hyallela azteca*, 31%). Invertebrate abundance was greater as one moved from the tunnel to shallower regions, as was the likelihood of encountering high density patches of prey. In the tunnel, mean biomass of invertebrates in benthic samples was 0.02 mg/cm^2 (SE = 0.01), with 4 of 10 samples devoid of invertebrates (range = 0−0.07 mg/cm^2). No snails appeared in snail or benthic samples. The cavern contained more benthic organisms than the tunnel ($P < 0.05$, Kruskal−Wallis test), with a mean biomass in benthic samples of 0.09 mg/cm^2 (SE = 0.02, range = 0.03−0.18 mg/cm^2) and an average of 95 snails/m^2 (SE = 18, mostly *Goniobasis clenchi*). The basin contained the most sediment-dwelling invertebrates (mean = 0.19 mg/cm^2, SE = 0.04, range = 0.01−0.42 mg/cm^2), which was greater than the tunnel but not the cavern ($P < 0.05$ and $P > 0.10$, respectively, Kruskal−Wallis test). Snail densities in the basin were lower than in the cavern (mean = 18 snails/m^2, SE = 5.3, t-test, $P < 0.01$). All organisms collected are potential prey for American eels (e.g. Tesch 1977) as are juveniles and even adults of many of the 15 other fish species that occur in the spring. Potential prey fishes seldom occurred in the cavern or tunnel.

Discussion

Patterns of Distribution and Abundance

Diel patterns of distribution of American eels in Vortex Spring suggest movement among regions that was size dependent. In the basin, numbers at night increased due to (1) movement of eels from the cavern and tunnel up into the spring basin and (2) emergence of eels hidden in the basin during the day. The relative contribution of the latter process is difficult to determine; some eels were probably buried in the basin during the day, as suction dredge operators occasionally dredge

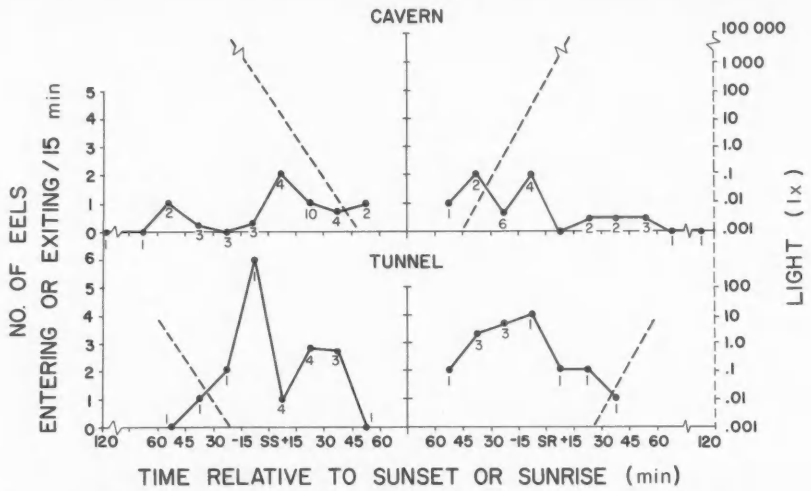


FIG. 6. Twilight changeover observations of American eels in Vortex Spring, based on diver observations of animals exiting and entering cavern and tunnel mouths. Solid circles represent mean number of eels observed during each 15-min interval, with number of observations for each time period shown below the circle. One observation was also made for each of the 15-min periods between 60 and 120 min before sunset and after sunrise, during which eels neither exited nor entered the cavern. Right vertical axis and broken, angled lines are average maximum illumination levels during twilight, as measured every 5 min at the cavern mouth (seven dusks, five dawns). For light curves at tunnel mouth, measured daytime maximum illumination was used as an initial value, and time course of light change at the tunnel mouth was assumed to parallel that at cavern mouth. SS = sunset, SR = sunrise.

eels out of the sediments (J. Van Wagoner, Vortex Spring, FL, pers. comm.). In the cavern, constant density but increased biomass at night probably resulted from emigration of small eels to the basin and immigration of larger eels from the tunnel. Reduction in numbers and biomass in the tunnel at night is largely a function of emigration.

Nocturnal reduction in numbers of small eels (<30 cm) in the tunnel and cavern and increase in numbers of small eels in the basin suggest a greater tendency by small eels to emigrate to the basin. The frequency distribution of size by depth indicates that more small animals occur in shallower water and more large animals occur in deeper water. Divers who have penetrated the Vortex tunnel to greater depths than our study area report that the largest eels occur at greater depths (J. Van Wagoner and D. J. Dockery, Vortex Spring, FL, pers. comm.).

The distribution of large animals at night suggests that large residents of the tunnel and cavern tend to remain in the cave regions at night, or that eels in the tunnel and cavern at night are large individuals that have moved up the tunnel from regions deeper than our sampling area. Large animals could be less mobile because of territoriality and behavioral dominance over smaller eels. During four of five observations of agonistic behavior, a larger eel bit a smaller eel, after which the smaller eel fled. On the one occasion when a smaller eel was the aggressor, the larger eel did not flee. We have frequently observed similar, size-dependent interactions in aquaria (see also Tesch 1977). Larger animals may be more successful territory holders, causing smaller animals to range more widely when foraging. The inverse relationship between eel size and time of reentry into the tunnel at dawn could similarly result from smaller eels moving greater distances from the tunnel in the evening, and requiring more time to return to the tunnel the following morning.

If prey are more abundant in the basin, why would behaviorally dominant, large eels not migrate there? If sufficient trophic resources occur in the cavern, large eels could avoid the energetic costs of migration by remaining there. Benthic prey in the cavern, although less dense than in the basin, were still abundant. Snails were most abundant in the cavern and are probably more available to large eels (e.g. Lammens et al. 1985). Fish prey, either red eye chub (*Notropis harperi*) which migrate into the cavern and tunnel in the evening, or even small eels, could also provide an occasional meal for large eels. Size-related differences in eel distribution by day and night could therefore reflect an interaction between behavioral dominance and the distribution of appropriate food types. However, large eels would also appear more sedentary if they had longer intervals between foraging bouts, which could presumably result from lower metabolic rates and larger meals.

Overall population estimates for the entire cave-spring system indicated substantial increases in numbers, density, and biomass at night (Table 1). Such increases could reflect emergence of eels from the sediments, particularly in the basin. Densities in the cavern and tunnel, where counts were absolute rather than based on a transect subsample, decreased slightly by night. These decreases could be accounted for by movement from the cavern and tunnel up to the basin by some eels, as discussed above. Despite the uncertainty associated with either population estimate, these are the first abundance measures for a population of American eels that account for all size classes (e.g. Bozeman et al. 1985).

Determinants of Activity Cycles

Factors thought to influence general activity periods and times of onset and cessation of activity in fishes include light,

hunger, prey availability, and ontogeny (Helfman 1986a). In Vortex Spring, the influence of light was suggested by several observations. Changeover activity at dusk increased when light levels began to diminish late in the afternoon. At dawn, activity at the tunnel mouth was maximal around sunrise and then tapered off, suggesting avoidance of daytime light levels in the basin and cavern. The actual illumination levels at which movement occurred also indicate that activity was influenced by light. Cessation and onset of activity occurred at both tunnel and cavern mouths at around 10–100 lx, suggesting an illumination threshold above which activity is inhibited. A light level of 10–100 lx, which commonly occurs about 10 min after sunset and before sunrise, has been correlated with changes in activity of a number of diurnal and nocturnal fishes (Hobson 1972; Helfman 1981; Helfman et al. 1982; Howick and O'Brien 1983).

Possibly superimposed on light-regulated phenomena are effects of endogenous rhythms. Laboratory investigations suggest that endogenous factors such as circadian rhythms can determine the onset and cessation of activity in fishes (Thorpe 1978; Schwassman 1980). Eels moved out of the tunnel from regions of total darkness in midafternoon. Such movement occurred before illumination underwent afternoon reductions, even in the illuminated regions of the tunnel. These individuals could have responded to an endogenous, circadian cue (e.g. Edel 1976). However, such responses may still be constrained by inhibitory light levels: only 3 of 27 eels moved up the chimney before the 100-lx level was reached there, and only 3 of 16 returning animals did so after the 100-lx level was exceeded at dawn.

Hunger could also stimulate migration out of the tunnel. Anguillid eels feed on essentially any prey small enough to be swallowed whole or torn apart, including oligochaetes and amphipods (Facey and LaBar 1981; Helfman and Clark 1986). The tunnel contained little food during day or night. Eels moving up the tunnel during the 3–4 h before sunset could have been responding to hunger cues and could have foraged in the relatively rich sediments of the basin or on the more abundant snail resources of the cavern. Although no statements can be made concerning the possible influence of prey availability on eel activity on a diel basis, movements did correlate with spatial differences in prey abundance. At dusk, eels migrated to regions where potential benthic prey were more abundant. Twilight migrations between daytime resting and nighttime feeding areas are characteristic of nocturnal fishes in many habitat types (Hobson 1973; Helfman 1981, 1986a).

Both hunger and circadian cycles could also explain the return of eels to the tunnel during predawn hours. Animals that left the tunnel 2–4 h before sunset may have returned 2–4 h before sunrise, in response to an endogenous rhythm. Alternatively, early-returning eels could be those that fed successfully early and consequently returned to their "daytime" resting areas earlier than less successful foragers, although activity per se did not cease upon this presumed return (Fig. 4B). Recent work indicates an interaction among light, feeding, and endogenous rhythms: the time at which a fish feeds can reset its internal clock (Speiler and Noeske 1984). These possible alternatives could be tested by comparing stomach fullness of returning animals versus those still in the shallows throughout the predawn period. Lack of a difference in stomach fullness would support the endogenous control postulate, which could be further tested by detailed observations of individuals through the diel cycle.

Although most American eels in Vortex Spring were diurnally quiescent and nocturnally active, small (<30 cm) eels foraged among cavern sediments by day. An ontogenetic change from diurnal to nocturnal foraging has been found in several fishes that are predominately nocturnal when large (Helfman 1978, 1986a) and has been linked to improved light capture abilities with age (e.g. Walls 1942; W. N. McFarland and C. Wahl, Cornell University, Ithaca, NY, pers. comm.).

Variability of Twilight Changeover

Although changeover occurred at dusk and dawn, peaks in directional movement were not well defined, particularly at dusk. Some of this variation may result from ambivalence in individuals changing over early relative to the rest of the population, as suggested by presunset and presunrise peaks in nondirectional activity and as found in many other fishes (e.g. McFarland et al. 1979; Helfman 1981). However, variability in timing of changeover encompassed much more than limited presunset and presunrise intervals. Initiation of activity at the tunnel entrance extended over a 5-h period from SS − 4 to SS + 1 h. Cessation of activity (=number of eels entering the tunnel) lasted 8 h, from SR − 7 h to SR + 1 h. In addition, some diurnal activity occurred in all size classes. Some of this variability may result from the weak, "twilight" nature of light near the tunnel mouth during the day. An inhibitory light threshold of approximately 100 lx could complicate distinctive changeover responses in eels leaving the tunnel. Such eels would travel from a region of illumination below the proposed threshold into a region above the threshold. Hence, animals moving through the tunnel mouth may have ceased activity once inside the cavern or at least delayed movement to the basin until light in the cavern mouth fell below the inhibitory threshold.

Given this potential complication, changeover can be more conservatively described by activity at the cavern mouth, where animals would experience illumination changes more similar to surface events. Even at the cavern mouth, changeover was relatively prolonged, lasting approximately 2 h at both sunset and sunrise. The only other nocturnal, freshwater fishes for which extensive comparative data are available are the golden shiner (*Notemigonus crysoleucas*), brown bullhead (*Ictalurus nebulosus*), rock bass (*Ambloplites rupestris*), and black crappie (*Pomoxis nigromaculatus*) (Hall et al. 1979; Helfman 1981). All of these fishes also show considerable variability in changeover times, particularly when compared with night-active, tropical marine species (Helfman 1981, 1986a).

Previous treatments of twilight activities in fishes have emphasized comparisons of variability in timing among groups and possible determinants of such variation. Diurnally active, temperate freshwater fishes appear to undergo more variable changeover than do tropical marine species (Helfman 1981, 1986a). Although less well studied, nocturnal fishes show the same general pattern (McFarland et al. 1979; Helfman 1981, 1986a; Helfman et al. 1982). Postulated determinants of these patterns include relative twilight length, water clarity, visual physiology, and intensity of twilight predation.

Recent work in temperate and arctic marine environments (Hobson et al. 1981; Hobson 1986; reviewed in Helfman 1986a) shows extended activity at twilight. Latitudinal influences associated with increasing twilight length at higher latitudes may result in greater changeover variability than at lower latitudes. American eels in Vortex Spring occur at a

latitude intermediate to that of other investigations, yet show extreme variation in their changeover activities.

With regard to other possible influences on changeover variability, Vortex observations do not bear out predictions that temperate freshwater fishes in water as clear as that found on coral reefs will necessarily show more distinct, less variable changeover activities than in turbid temperate systems (Helfman 1981). The possible influences of predation intensity and ontogeny of visual capabilities are difficult to separate, as susceptibility to predation generally decreases with increasing body and eye size (e.g. Werner and Gilliam 1984; Helfman 1986b). Most eels in Vortex are too large to be highly vulnerable to the resident piscivores (bowfin (*Amia calva*), chain pickerel (*Esox niger*), largemouth bass (*Micropterus salmoides*)). Low predation pressure in Vortex may be reflected in the (1) high temporal variability in twilight activities and (2) lack of distinctive corridors of movement during twilight migrations (Helfman 1981, 1986a). Understanding the factors determining variability of twilight activities in American eels would require comparisons of the behavior and visual physiology of different size eels with respect to light-regulated activities, combined with manipulation of predation intensity on various size classes. Complicating all of these explanations is the possibility that activity cycles are under genetic control. Eels breed panmictically (Williams and Koehn 1984; Avise et al. 1986). Thus, variability in changeover timing could be a species characteristic, independent of local environmental influence. Comparative studies of different eel populations would be necessary to establish the occurrence of local differences in activity cycles.

Whether the activity patterns observed here are characteristic of nocturnal freshwater fishes in general, characteristic of American eels, or just unique to eels in Vortex Spring is unknown. All are possible, given the lack of comparative information on cave-dwelling fishes that migrate between subterranean and surface areas, on anguillid eel behavior in the field, and on the behavior of most nocturnal freshwater fishes.

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A Conceptual Genetic Fitness Model for Fisheries Management[1]

Anne R. D. Kapuscinski[2] and James E. Lannan

Department of Fisheries and Wildlife, Oregon State University, Marine Science Center, Newport, OR 97365, USA

Kapuscinski, A. R. D., and J. E. Lannan. 1986. A conceptual genetic fitness model for fisheries management. Can. J. Fish. Aquat. Sci. 43: 1606–1616.

A generalized model for managing fisheries to maintain reproductive fitness is proposed. The fitness of an individual within a stock is its number of successful progeny, where successful means survival to the reproductive stage. The fitness of a stock is a probability distribution (W) described by its mean ($\bar{W}$) and variance (V_w). An example of equations, derived from a variance effective number, expresses the distribution as a function of phenotypic variables (including abundance, immigration, and age structure) which could be estimated in fishery surveys. Management of a stock as a self-perpetuating system requires maintenance of its probability distribution at a sufficiently broad level, estimated from reference generations, to allow it to perpetuate in the face of fishing and natural mortality in a changing environment. Modification of the distribution by exploitation or management actions imposes the risk of reduction of genotypic diversity in the stock. Selection of appropriate reference generations requires careful attention to biological detail. Application of the model is illustrated by computing the effective number for fictitious stocks with different life histories.

Les auteurs proposent un modèle généralisé de gestion des pêches visant à maintenir l'aptitude reproductive. L'aptitude d'un individu d'un stock donné est représentée par l'importance de sa progéniture ayant survécu jusqu'au stade de la reproduction. L'aptitude d'un stock est la distribution des probabilités (W) décrite par sa moyenne ($\bar{W}$) et sa variance (V_w). On donne en exemple des équations, obtenues à partir d'une variance effective, qui expriment la distribution comme une fonction de variables phénotypiques (abondance, immigration et structure des âges) qui peuvent être estimées au cours de relevés des pêches. La gestion d'un stock en tant que système s'autoperpétuant exige le maintien de sa distribution de probabilités dans une gamme suffisamment large, estimée à partir de générations de référence, afin de lui permettre de se maintenir face à la mortalité par la pêche et à la mortalité naturelle dans un environnement changeant. La modification de la distribution par mesures d'exploitation ou de gestion fait apparaître le risque d'une réduction de la diversité génotypique. Le choix de générations de référence appropriées exige de porter une grande attention aux détails biologiques. L'application du modèle est illustrée par le calcul du nombre effectif de divers stocks fictifs d'antécédents variés.

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Perhaps no questions in contemporary fisheries science have stimulated more debate than those which relate to the long-term genetic implications of fisheries management and exploitation. In his eloquent review of the subject as it relates to the management of Pacific salmon fisheries, Larkin (1981) noted that "it is rather surprising that there is currently no explicit working hypothesis of population genetics in the salmon management kit box." The lack of a conceptual framework is a primary constraint to the integration of genetic considerations into stock management; it is not unique to salmon but is a general concern in fisheries science and, indeed, to the entire fields of natural resource management and gene conservation.

In reviewing the abundant literature addressing the many facets of the questions, we speculate that the pursuit of a conceptual framework may be more a matter of synthesis than one of developing new theory. Therefore, we have explored alternatives for assembling a conceptual framework drawing on existing principles of population genetics and population biology, and proposed a conceptual and quantitative model for the management of Pacific salmon fisheries based on the genetic concept of fitness (Kapuscinski and Lannan 1984).

In the course of applying the salmonid model to computer simulations with various species of Pacific salmon, we have revised and refined the conceptual and quantitative derivations. We are now prepared to propose a generalized genetic fitness model for fisheries management that has the following features.

(1) It can be applied to any species because it provides for appropriate consideration of life history patterns, reproductive strategies, and the like.

(2) It is a phenotypic model. The quantitative inputs for the conceptual model, including stock abundance, age structure, and immigration, are phenotypic variables that can be estimated or measured directly. Determination of genetic properties of the stock (e.g. gene frequencies, heterozygosity, etc.) are not required.

(3) The model output is the number of reproducing adults required in a given breeding season to insure the maintenance of the probability distribution of fitness for the particular life

[1]Oregon Agricultural Experiment Station Technical Paper No. 7006.

[2]Present address: Department of Fisheries and Wildlife, University of Minnesota, 200 Hodson Hall, 1980 Folwell Avenue, St. Paul, MN 55108, USA.

history of the stock.

(4) The functional relationships of the model can stand alone or can be linked to other predictive models of population dynamics.

(5) The model permits substantial management flexibility in assessing the genetic status of stocks relative to management goals. Thus, it is equally applicable to managing the harvest of surplus production on the one hand and to stock rehabilitation on the other.

Rationale for a Fitness Model

Virtually all contemporary approaches to natural resource management are directed toward a common goal: perpetuation of the resource. This consideration necessitates that management insures an adequate number of reproducing adults in breeding populations. In recent years, there has been proliferation of quantitative methods intended to estimate escapements required to provide a sustained harvest based upon this principle. Examples of contemporary efforts to apply the principles of population biology to fisheries management are provided by Schaefer (1954), who described the relationship between surplus production and stock biomass, and Ricker (1954), who described the relationship between spawners and recruits. These quantitative models do not allow the assessment of genetic changes caused by management and exploitation. This is a serious limitation because even if the model predictions result in adequate escapement and subsequent recruitment in the next generation, one cannot exclude the possibility that the stock experiences a net reduction in diversity of genetic information that will limit its opportunities for adaptation in a dynamic environment in subsequent generations.

The unifying genetic concept related to the long-term probability of survival of breeding populations is termed fitness (or adaptive value, selective value, or capacity). Fitness is defined genetically as the contribution of an individual in a breeding population to subsequent generations. Mean fitness is defined as the average fitness of a population. It is important to recognize that both populations and individuals within populations exhibit fitness.

If fitness is the genetic property related to the perpetuation of a breeding population, then maintaining fitness must be the goal of fisheries management. Larkin's (1970) definition of a stock as "a self perpetuating system" is consistent with this goal.

The fitness of an individual is the phenotypic expression of all genetic and environmental circumstances affecting survival and reproduction encountered through its life history. Therefore, we assume that fitness reflects genetic variation, because it is a quantitative character with both genetic and environmental sources of variance. This assumption must be valid for there to be concern about the genetic implications of fisheries management and exploitation. Otherwise, neither activity will influence the long-term genetic health of stocks; in fact, stocks would not even respond to natural selection. However, unless this assumption is proven invalid it is reasonable to consider that fitness has genotypic variance because the literature is replete with inference that this is the case.

Fitness is a phenomenon that has uncertainty associated with it because the sequence of environments that an individual might encounter cannot be predicted. However, its variation can be described by a probability model (Hillier and Lieberman 1974). Therefore, it is appropriate to model fitness as a random variable with a continuous probability distribution for a large number of individuals in the stock. Thus, one may conceptualize a probability distribution of fitness (W) for an entire stock, with a corresponding mean ($\bar{W}$) and variance (V_w) of the distribution. For a given species, this distribution is determined by reproductive strategies and life history patterns, and reflects the genetic variation necessary for adaptation through the range of environments encountered by the stock.

The genetic concept of fitness can provide a conceptual framework for fisheries management by restating the management goal to be the maintenance of the probability distribution of fitness over time. It is appropriate to consider a probability distribution of fitness rather than simply a mean value. Although average fitness may be indicative of trends in stock abundance during a given period, the long-term survival of the stock may be more dependent upon the maintenance of an adequate degree of variance than only on the mean value of fitness. Because the phenotypic variance of fitness reflects both genotypic and environmental sources of variance, maintaining the variance of fitness at adequate levels should insure an adequate degree of genetic diversity to enable stocks to adapt to changing environmental conditions over time.

The contemporary literature is replete with references that emphasize the evolutionary significance of the variance of fitness. For example, Williams (1977) noted that the variability of individual fitness must be substantial and that only a few genotypes make an effective contribution to the next generation in populations of high-fecundity animals, e.g. many species of fish. Real (1980) proposed that natural selection maximizes a function containing terms for both V_w and $\bar{W}$, while Conrad (1983) argued that the variability of biological matter "is the *sine qua non* for the ability of organisms to cope with the uncertainty of the environment."

Conceptual Model

Let W equal the probability distribution of fitness (with characteristic $\bar{W}$ and V_w for a stock. Functional relationships that express the probability distribution can be expressed as a function of population variables that influence stock fitness. Let us assume that the stock under consideration is not likely to undergo any significant evolutionary change during the period in which management is applied. Then, the functional relationships for the model should address the principal mechanisms that can effect substantial changes in the diversity of genetic information in a stock in one to several generations. These include changes in size of the reproducing stock, immigration into the stock, the presence or absence of age structure in the reproductive stock, selection, and mutation. For a management model, it is not necessary to include functional relationships addressing the latter two variables. Mutation may be considered negligible in the short term, and the maintenance of the probability distribution of fitness through the course of natural selection is implicit in the management goal.

Figure 1 is an illustrative example of the functional relationship between the probability distribution and two population variables, number of reproducing individuals and age structure. The upper panel illustrates a number of hypothetical probability distributions of fitness for breeding individuals of a given age in a fictitious stock. Individual fitness is expressed as the number of successful progeny per parent where successful means survival to the reproductive stage. Note that these distributions

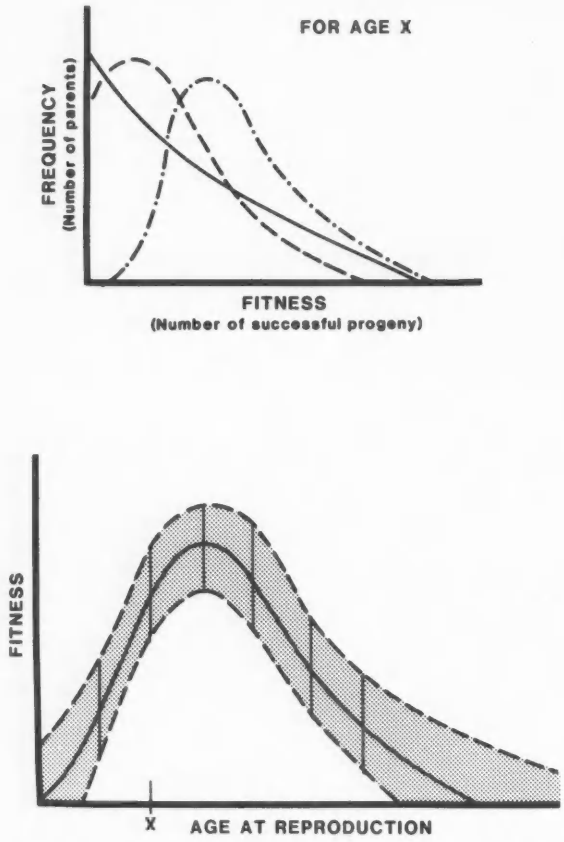

FIG. 1. (Upper) Three hypothetical probability distributions of fitness for breeding individuals of a given age in a fictitious stock. (Lower) Fitness as a function of reproductive age in the stock. The solid curve represents $\bar{W}$ for each age class. The shaded area represents an integration of the values for $\bar{W}$ and V_w over all reproductive ages. Additional explanation is provided in the text.

might also represent a stock without age structure (i.e. generations do not overlap) or a stock with age structure in which fitness is not age dependent. The area under each curve represents the total number of reproducing individuals present in the stock after fishing and natural mortality.

The probability distribution of fitness as a function of all the ages present in the fictitious stock is illustrated in the lower panel of Fig. 1. This graph was generated by integrating the values for $\bar{W}$ and V_w (from an appropriate fitness distribution of the types represented in the upper panel) over all ages (Fig. 1, lower). The genetic objective can then be restated as maintaining the variance (shaded area) around the entire curve in the lower panel.

If there is immigration into a stock, the lower panel could be expanded into a response surface expressing fitness as a function of both age and immigration.

If exploitation results in modifying the probability distribution (upper panel) for one or more age classes in the stock, the variance (shaded area) in the lower panel is reduced. Reduction of the phenotypic variance of fitness imposes the risk of attendant reduction of genotypic diversity in the stock. Maintaining the phenotypic variance of fitness minimizes this risk.

Application of the conceptual model to fisheries management necessitates deriving quantitative functional relationships. Although it would be desirable to determine the probability distribution of fitness in quantitative terms, this may be impractical or impossible in most situations. However, in attempting to rationalize a management concept based on fitness, we have concluded that it is not essential to assign absolute numerical values directly to the distribution because the real concern is how it changes with time.

Let W equal the probability distribution of fitness (with a characteristic $\bar{W}$ and V_w) in past breeding seasons and W' equal the probability distribution of fitness in the breeding seasons of management concern. Assuming that W is at a value consistent with management goals, conservative management dictates that it should not decrease in subsequent breeding seasons, thus:

$$(1) \quad W = W'.$$

In some management situations, it may be more appropriate to express this relationship as

$$(2) \quad W \leqq W'.$$

The functional relationships $W = f$(population variables) and $W' = f$(population variables') can be substituted into equation (1) and the equation rearranged to solve for any one population variable, assuming that numerical values for the remaining variables can be estimated.

In articulating a fitness model for Pacific salmon, we stated the genetic objective to be to maintain the variance of fitness. However, in extending the concept to a generalized fisheries management model, we now propose that the objective is to maintain the probability distribution of fitness. Because the probability distribution will be different for different life history patterns, this broader objective will simplify applying fitness models to different species and thus result in a more comprehensive and conservative genetic objective for stock management.

Functional Relationships

This section describes an example of functional relationships for a fitness model and demonstrates adjustments of the model to accommodate different life histories.

The functional relationships represent a second approximation of functional relationships consistent with the objectives of stock management. The first approximations (Kapuscinski and Lannan 1984) have been revised to include improved and more general expressions for the incorporation of age structure. These functional relationships are presented as one example of quantitative expressions for the conceptual model. We anticipate that new derivations will result as the conceptual model becomes more comprehensive.

The functional relationships for the model are based on the concept of a variance effective number. Relating any genetic property of a population to actual population numbers is complicated because not all individuals in a population, not even all sexually mature individuals, necessarily become parents (Falconer 1981). The concept of effective population size was introduced by Wright (1931) to reduce the complicated breeding structure of real populations to the mathematically equivalent and simpler case of an idealized population. According to theory, two related processes, inbreeding and random drift,

contribute to an average increase in homozygosity within a population of finite size. Thus, two definitions (Kimura and Crow 1963) of the effective population number, N_e, must be considered: (1) the inbreeding effective number, $N_{e(f)}$, is the size of an idealized population that has the same rate of inbreeding as the observed population and (2) the variance effective number, $N_{e(v)}$, is the size of an idealized population that has the same amount of random gene frequency drift as the observed population. Either effective number offers the additional advantage of incorporating the probability of increasing homozygosity, thus eliminating the requirement of estimating homozygosity in managed populations.

The two effective numbers are equivalent for populations that remain constant in size over time, and are approximately equal for populations whose periods of increase and decline tend to negate one another over time (Crow and Kimura 1970). However, for small populations that are in decline, especially those that are in danger of extinction, gene frequency drift results in greater homozygosity than does inbreeding (James Crow, University of Wisconsin, pers. comm.). Thus, although one can derive a functional relationship using either $N_{e(f)}$ or $N_{e(v)}$, conservative management seems to favor the latter.

An equation for $N_{e(v)}$ can be derived that incorporates population size, immigration, age structure, and the variance of fitness. If we define genetic risk as the probability of a reduction in the adaptability of a stock to its habitat due to a reduction in genetic variation, then the final expression for $N_{e(v)}$ will be a measure of genetic risk. It will not be equivalent to the $N_{e(v)}$ presented in the literature of population genetics (Crow and Kimura 1970). Recognizing that $N_{e(v)}$ is an abstraction, the reason for including it in the quantitative expressions is to permit the unification of the principal mechanisms affecting stock fitness. Then we can use the functional relationship $N_{e(v)} = f$ (population variables) for the conceptual model if we note that the probability distribution of fitness is a function of $N_{e(v)}$.

The equation for $N_{e(v)}$ will be used to satisfy the following management objective. If $N_{e(v)}$ is calculated for a stock during a breeding interval when stock abundance satisfied management goals, then conservative management dictates that this value should not decrease in subsequent breeding seasons, thus:

$$(3) \quad N_{e(v)} \leqq N'_{e(v)}$$

where the prime notation distinguishes the breeding seasons in present time from the reference breeding seasons. Functional relationships for $N_{e(v)}$ and $N'_{e(v)}$ can be substituted into equation (3) and the equation rearranged to solve for any one population variable, assuming that numerical values for the remaining variables are available.

Population Size

Because $N_{e(v)}$ is the size of an idealized population, the simplifying properties of an idealized population must be considered in the present derivation. Thus, we consider an observed population with the following properties: (1) mating is at random, (2) the sexes are separate but the numbers of each sex need not be equal, (3) self-fertilization does not occur, (4) migration into the population does not occur, (5) parents reproduce at only one age (generations are discrete), (6) the population is composed of diploid individuals, (7) the number of offspring per parent contributed to the next generation can vary.

The variance effective number for this population is

$$(4) \quad N_{e(v)} = \frac{N_{t-1}\bar{k}}{1 + \frac{V_k}{\bar{k}}}$$

where N_{t-1} is the number of breeding individuals in breeding season $t-1$ and $\bar{k}$ and V_k are, respectively, the mean and variance of successful gametes (i.e. gametes that survive to become parents in the next generation) contributed per parent (Kimura and Crow 1963; Crow and Kimura 1970). The variance effective number can be weighted for differential contributions of male and female parents by calculating N_e^* for females and N_e^{**} for males and combining them according to the formula of Wright (1931):

$$(4a) \quad N_{e(v)} = \frac{4N_e^* N_e^{**}}{N_e^* + N_e^{**}}.$$

Because $\bar{k}$ and V_k express a relationship between parents in generations $t-1$ and t, $\bar{k}$ and V_k are proportional to $\bar{W}$ and V_w. We use V_k in place of V_w without solving the proportion in the present discussion because we will ultimately equate $N_{e(v)} = f(V_k)$ to $N'_{e(v)} = f(V'_k)$, as suggested in equation (3). Similarly, $\bar{k}$ replaces $\bar{W}$ and is estimated as (Crow and Kimura 1970)

$$(5) \quad \bar{k} = \frac{2N_t}{N_{t-1}}$$

based on the numbers of parents at t and $t-1$.

Because equation (4) applies to the observed population we describe above, it does not apply to cases where immigration or age structure occur. The next step in our synthesis must therefore address immigration and age structure.

Immigration

Immigration reduces the probability of random drift among subpopulations. The simplest model for migration, the Island Model (Wright 1951), assumes that the immigrants into a subpopulation are a random sample of the entire population. Thus, the migrants have a gene frequency equal to that for the whole population.

The Island Model can be applied to the present case by assuming the stock to be a subpopulation. Let M be the fraction of the stock (subpopulation) replaced by migrants in a generation. The probability that neither of two genes uniting in a zygote has been exchanged for a migrant gene is $(1-M)^2$. Because random drift of gene frequencies (and the corresponding loss of genetic variation) within stocks decreases as migration increases among them, inclusion of migration in equation (4) should result in an increase in $N_{e(v)}$ when M increases. The appropriate expression for $N_{e(v)}$ under these conditions is

$$(6) \quad N_{e(v)} = \frac{N_{t-1}\bar{k}}{1 + \frac{V_k}{\bar{k}}(1-M)^2}$$

based on the equation presented by Crow and Kimura (1970) for the rate of inbreeding as a function of M and $N_{e(v)}$.

The assumptions of the Island Model can be relaxed to correct for migration between subpopulations that are related due to exchange of genetic information in previous generations (Crow and Kimura 1970). If r is the correlation of gene frequencies between immigrants and the receiving subpopulations, $M(1-r)$ is substituted for M in equation (6).

Age Structure

When individuals reproduce at two or more ages, a population may be composed of individuals representing two or more cohorts in a given calendar period. In a management sense, this period must include the interval during which fishing mortality and reproduction occurs. We find it useful to define a breeding season as an interval of management concern and to consider all cohorts represented during that interval in the calculations of N_{t-1}, $\bar{k}$, and V_k in equation (6).

We previously suggested using a term for the average age of reproduction, τ, to adjust $N_{e(v)}$ for overlapping generations (Kimura and Crow 1963). Because calculations of τ over time involve the assumption of stable population size and because of inconsistencies between the derivations for τ and $N_{e(v)}$, we have replaced τ with age-specific values for the input variables in equation (6). This approach treats each reproductive age class as a subset of the entire stock such that $N_{e(v)}$ or W for the whole stock is a weighted sum of the values for each age.

Incorporation of age structure into equation (6) requires estimating the abundance of parents $N_{x,t-1}$, in each cohort, where x is the age at reproduction. Then the abundance of all parents at $t-1$ in equation (6) is

$$(7) \quad N_{t-1} = \sum_x N_{x,t-1}.$$

Likewise, the contribution of each cohort to the average number of successful gametes must be considered. The appropriate expression is the weighted sum

$$(8) \quad \bar{k} = \frac{\sum_x \bar{k}_x N_{x,\, t-1}}{N_{t-1}}$$

where $\bar{k}_x$ is the average number of successful gametes produced by parents of age x.

Alternatively, equation (5) for $\bar{k}$ can be modified to

$$(9) \quad \bar{k} = \frac{2 \sum_n N_{x+n,\, t+n}}{N_{t-1}}$$

a form that may be calculated from estimates of population abundance at breeding season $t-1$ and $\Sigma_n\, t+n$ where the range of values for n is determined by the reproductive life history of a given stock. For example, if there is annual breeding and the progeny produced by parents at $t-1$ are sexually mature at ages 1–5, then the range of n is 0–4; the numbers of reproductive progeny are estimated for five consecutive breeding seasons following $t-1$.

Finally, the variance of successful gametes can incorporate age structure by computing a weighted sum of the variances of each cohort. This requires a few intermediate steps based on statistical concepts. If $\sigma^2_{k,x}$ is the true variance of k for fish of age x and $s^2_{k,x}$ is the variance of k for fish of age x within a given stock, then $s^2_{k,x}$ for one stock is a sample of $\sigma^2_{k,x}$, the true variance for all stocks of the species:

$$(10) \quad \sigma^2_x = s^2_{\text{within stocks}} + s^2_{\text{among stocks}} \qquad s^2_x < \sigma^2_x.$$

The definition of $N_{e(v)}$ implies that it is a measure of the sampling bias in the transmission of genes from one generation to the next caused by the finite size of a stock. Thus, it is appropriate to make $s^2_{k,x}$ dependent on the sample or stock size. This requires removing the Gaussian correction from the variance formula and is accomplished by

$$(11) \quad s^2_{k,x} = \frac{\sigma^2_{k,x}\,(N_x - 1)}{N_x}$$

where N_x refers to breeding season $t-1$.

If it is assumed that $s^2_{k,x}$ at one age (x) is independent of $s^2_{k,y}$ at any other age (y) present in the reproductive stock, then

$$(12) \quad \text{COV}_{k,xy} = 0$$

for all paired combinations of ages. Then, the contribution of each cohort to V_k is considered by computing the weighted sum

$$(13) \quad V_k = \frac{\sum_x s^2_{k,x} N_{x,t-1}}{\sum_n N_{x,t-1}} = \frac{\sum_x s^2_{k,x} N_{x,t-1}}{N_{t-1}}.$$

When expression (12) is unacceptable, the summation in the numerator of expression (13) must include the appropriate covariances.

Substitution of expressions (7), (8) or (9), and (13) for N_{t-1}, $\bar{k}$, and V_k, respectively, into equation (6) provides a functional relationship between $N_{e(v)}$ and population size, the mean and variances of stock fitness, age structure, and migration. The solution of equation (6) requires the following census data: estimates of stock abundance at $\Sigma_n\, t+n$ and $t-1$; the proportion of reproducing adults at $\Sigma_n\, t+n$ and $t-1$; the number of migrants into the stock in breeding season $t-1$; the age composition of reproducing adults at $\Sigma_n\, t+n$ and $t-1$; and estimates of age specific variances of successful gametes ($s^2_{k,x}$) at $t-1$.

While the first four items can be estimated from data monitored in fisheries management, measurement of $s^2_{k,x}$ may require carefully designed field sampling of marked progeny (e.g. with coded wire tags) over $\Sigma_n\, t+n$ breeding seasons. An alternative method of estimating $s^2_{k,x}$ requires a knowledge of the probability distribution of k and estimates of $\bar{k}_x$ at each age x (see Fig. 1). For some probability distributions (e.g. Poisson, gamma, exponential, and binomial distributions), the variance can be expressed as a function of the mean. If the probability distribution of k at age x fits this condition, then

$$(14) \quad s^2_{k,x} = f(\bar{k}_x).$$

However, estimation of $\bar{k}_x$ still may require sampling of marked progeny.

Application of The Quantitative Model to Stock Management

Equation (6), which contains substitutions from equations (7), (8), and (13) for age structure, is in a form which can be substituted into equation (3). Thus, the conceptual model can now be applied to real management situations for stocks with migration and age structure. This application would typically involve the following stepwise process.

(1) The historical records for a stock of concern would be surveyed, and successive breeding seasons, $t-1$ and $\Sigma_n\, t+n$, would be selected from an era when the stock would be considered to have $N_{e(v)}$ consistent with the management goal. The terms N_{t-1}, (equation (7)), $\Sigma_n\, N_{x+n,\, t+n}$, M, and V_k (equation (13)) would be compiled for the selected interval.

(2) The immediate management objective is to determine the number of breeding individuals required in the present (or next) breeding season to insure that $N_{e(v)}$ (thus W) does not decrease.

TABLE 1. Number of pink salmon spawners for three generations ($g = 3$). Data correspond to curves in Fig. 4.

Abundance trend	N_1	N_2	$N_3 = N_{t-1}$
Declining	5000	2000	1000
Increasing	200	600	1000

This is done by evaluating the reproductive individuals for abundance, age structure, and immigration in the generation interval of immediate concern. These data would provide numerical values for N'_{t-1}, M', and V'_k, where the prime notation is intended to distinguish these properties in present time from the corresponding terms for the reference generations. The variable for which we will ultimately solve is $\Sigma_n N_{x+n,\,t+n}$.

(3) Consistent with equation (3), we now equate $N_{e(v)}$ for the reference generation to $N'_{e(v)}$ for the present generation, giving

$$(15)\quad \frac{N_{t-1}\bar{k}}{1+\frac{V_k}{\bar{k}}(1-M)^2} = \frac{N'_{t-1}\bar{k}'}{1+\frac{V'_k}{\bar{k}'}(1-M')^2}$$

Substituting $2\Sigma_n N_{x+n,t+n}/N_{t-1}$ for $\bar{k}$ and $2\Sigma_n N'_{x+n,t+n}$ for $\bar{k}'$ (equation (9)) and rearranging terms, we arrive at

$$(16)\quad \sum_n N'_{x+n,t+n} = \frac{\sum_n N_{x+n,t+n}\left[1+\left(\frac{N'_{t-1}V'_k}{2\sum_n N'_{x+n,t+n}}\right)(1-M')^2\right]}{1+\left(\frac{N_{t-1}V_k}{2\sum_n N_{x+n,t+n}}\right)(1-M)^2}.$$

Equation (16) would be appropriate for species with age structure. A similar stepwise process would be employed for species with discrete generations; however, the expression $\Sigma_n N_{x+n,t+n}$ reduces to N_t in this case.

An Example

We have computed $N_{e(v)}$ while different combinations of variables were held constant using data for fictitious chum salmon (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*) stocks. These species were selected because they have different life histories; the former typically has four reproductive age classes (i.e. overlapping generations), whereas the latter has only one reproducing age class (i.e. generations are discrete). The $\bar{k}_x$ values for the chum salmon stock were held constant in all the calculations. Unless otherwise specified, the reproductive ages and the age distribution in the chum stock were age 2 (5%), age 3 (50%), age 4 (35%), and age 5 (10%). Also, the chum and pink stocks were declining in abundance ($\bar{k} \leqq 1$). The values used to simulate three possible abundance trends in a pink salmon stock (see Fig. 5) are given in Table 1.

In addition to the properties of stocks listed as assumptions for equation (4), we assume that the stocks have the following properties: (1) migration into the populations may occur; (2) for each reproductive age, the frequency distribution of successful gametes per parent is described by a Poisson distribution (see Fig. 1, upper); (3) there is a linear increase in the average number of successful gametes per parent ($\bar{k}_x$) as age increases; and (4) there are no sex-specific differences in the frequency distribution of successful gametes per parent.

Equation (6) is the expression for $N_{e(v)}$ for these stocks. For the chum salmon stock, which has more than one age class in the mating population, expressions (7), (8), and (13) are substituted into expression (6). The age-specific variances in equation (13) are assumed to be from Poisson distributions, thus:

$$(17)\quad s^2_{k,x} = \frac{\sigma^2_{k,x}(N_x - 1)}{N_x}$$

$$(18)\quad \sigma^2_{k,x} = \bar{k}_x.$$

For the pink salmon stock, in which generations are discrete (i.e. $t-1$ and t refer to breeding seasons for either an odd-year or an even-year stock), equation (5) is substituted into equation (6). Also,

$$(19)\quad V_k = \frac{\sigma^2_k (N_{t-1} - 1)}{N_{t-1}}$$

$$(20)\quad \sigma^2_k = \bar{k}.$$

Comparisons between the chum and pink stocks are presented in Fig. 2–5, showing $N_{e(v)}$ as a function of other input variables for the reference generation. As implied in equation (15), these figures also represent functional relationships for the present generation under management ($N_{e(v)}$); but this fact was omitted from the legends for simplification.

$N_{e(v)}$ as a Function of N_{t-1} for Constant $\bar{k}_x$

There is a linear increase in $N_{e(v)}$ as N_{t-1} increases when the immigration rate (M) is held constant (Fig. 2). The pink and chum stocks behave identically when $\bar{k}$ (i.e. $\bar{k}_2$) in the pink stock is equal to $\bar{k}$ weighted for all ages ($\bar{k}_2$, $\bar{k}_3$, $\bar{k}_4$, $\bar{k}_5$) in the chum stock. This result will occur in nature only if $\bar{k}_2$ for pink salmon is greater than $\bar{k}_2$ for chum salmon. If $\bar{k}_2$ for pink salmon is equal to $\bar{k}_2$ for chum salmon (i.e. $\bar{k}$ for the pink stock is less than $\bar{k}$ weighted for all ages in the chum stock), then the slope of the pink stock's line would be less than that of the chum stock. This means that a pink stock would require a larger escapement (N'_{t-1}) than a chum stock to maintain $N'_{e(v)}$ at a given value of $N_{e(v)}$.

A loss of reproductive ages in the chum stock results in a decrease in $\bar{k}$ and V_k (Fig. 2). This leads to an increase in the escapement (N'_{t-1}) required to maintain $N'_{e(v)}$ at a given value of $N_{e(v)}$. For example, if $N_{e(v)}$ for the reference generation is 200 and if the management goal is to maintain $N'_{e(v)}$ at this value, then the required N'_{t-1} is 450 for a chum stock consisting of ages 2 and 3 versus 350 for a chum stock consisting of ages 2–5.

There is a linear increase in $N_{e(v)}$ as N_{t-1} increases when age class structure and M are held constant for different values of V_k and $\bar{k}$ (Fig. 3). The pink and chum stocks behave identically

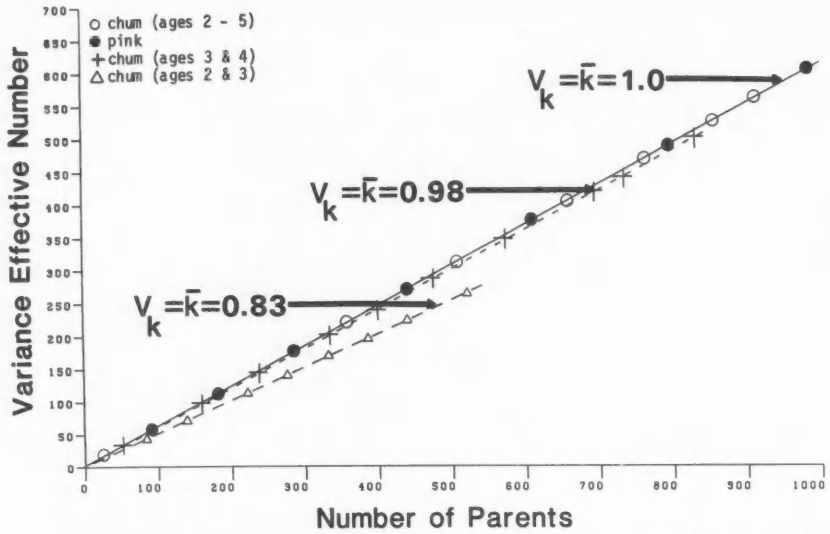

FIG. 2. Variance effective number, N_e, as a function of the number of parents (N_{t-1}) at breeding season $t-1$ and age structure (for constant values of age specific average number of successful gametes, $\bar{k}_x$) when immigration is held constant ($M = 0.2$).

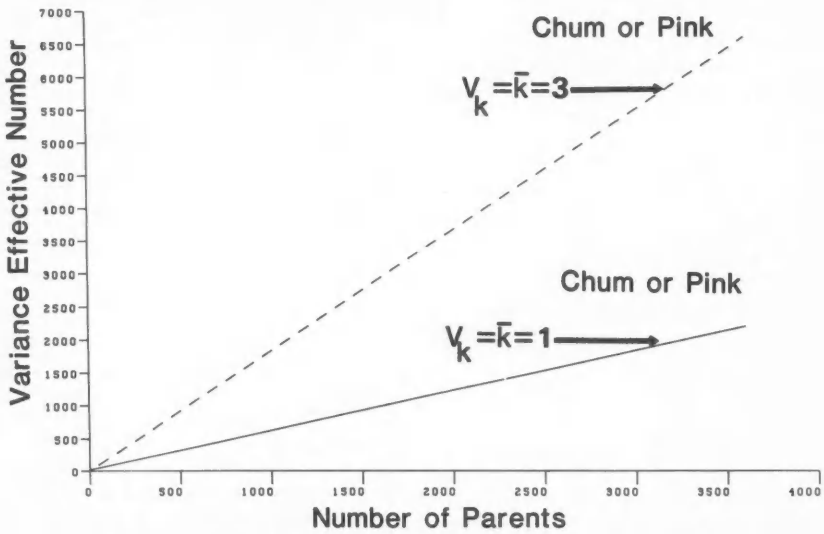

FIG. 3. Variance effective number, N_e, as a function of the number of parents (N_{t-1}) at breeding season $t-1$ and the variance of successful gametes (V_k) when age class structure and immigration are held constant ($M = 0.2$).

when $\bar{k}$ (i.e. $\bar{k}_2$) in the pink stock is equal to $\bar{k}$ weighted for all ages in the chum stock. The escapement (N'_{t-1}) required to maintain $N'_{e(v)}$ at a given value of $N_{e(v)}$ decreases as V_k and $\bar{k}$ increase.

There is a linear increase in $N_{e(v)}$ as N_{t-1} increases for different age class structures and values of M (Fig. 4). The pink and chum stocks behave identically for the same value of M when $\bar{k}$ in the pink stock is equal to $\bar{k}$ weighted for all ages in the chum stock. The escapement (N'_{t-1}) required to maintain $N'_{e(v)}$ at a given value of $N_{e(v)}$ decreases as M increases for a given age class structure.

In the chum stock, an increase in M counteracts the reduction in V_k caused by the loss of reproductive ages. The elevation and V_k value of the curve for ages 2 and 3 are lower than are the elevation and V_k value of the curve for ages 2–5 when M is held constant ($M = 0$). Thus, N'_{t-1} required to maintain $N'_{e(v)}$ at a given $N_{e(v)}$ is greater for ages 2 and 3 than it is for ages 2–5. Although V_k remains constant, the elevation of the curve for ages 2 and 3 increases when M increases to 0.4. Then, N'_{t-1} required to maintain $N'_{e(v)}$ at a given $N_{e(v)}$ is smaller for ages 2

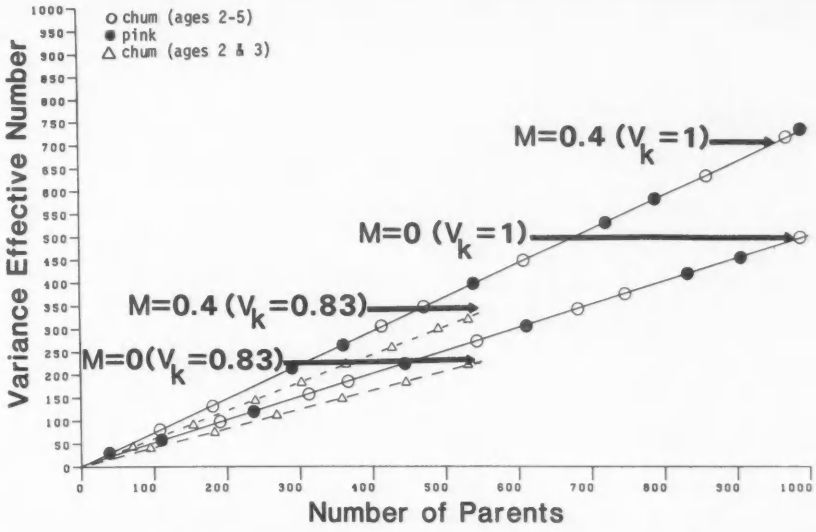

FIG. 4. Variance effective number, N_e, as a function of the number of parents (N_{t-1}) at breeding season $t-1$, migration, and different age structures (with constant values of age-specific average number of successful gametes, $\bar{k}_x$).

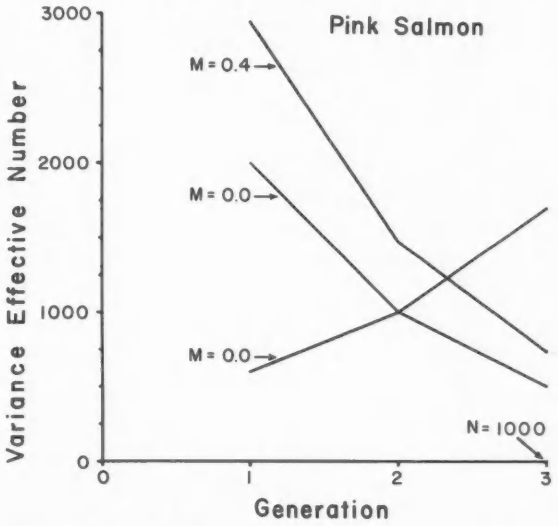

FIG. 5. Variance effective number, N_e, as a function of time, the trend in stock abundance, and migration rate when the number of pink salmon spawners at breeding season $t-1$ is held constant (N_{t-1} = 10000.

and 3 and $M = 0.4$ than it is for ages 2–5 and $M = 0$. If some mechanism other than a change in age class structure reduces V_k in a pink or chum stock, an increase in M will counteract the impact on $N'_{e(v)}$.

$N_{e(v)}$ as a Function of the Trend in Stock Abundance

The value of $N_{e(v)}$ at breeding season $t-1$ is different for stocks experiencing different trends in abundance when N_{t-1} (i.e. N at generation 3) is held constant (Fig. 5). At generation 3, $N_{e(v)}$ is much smaller for a pink stock declining in abundance than it is for a pink stock increasing in abundance. A 40% increase in M in the declining stock increases the value in $N_{e(v)}$, although it still is much smaller than $N_{e(v)}$ for the increasing stock. Thus, it may be difficult or even impossible to attain a given level of $N'_{e(v)}$ (determined by reference generation values or other management objectives) when a stock is experiencing a rapid decline in abundance. The escapement required to maintain $N'_{e(v)}$ at a given value of $N_{e(v)}$ will have to be much greater in a declining stock than in an increasing stock.

Assumptions

The functional relationships used in this example for age-structured salmon stocks assume a Poisson distribution for k at a given age, a constant value for each $\bar{k}_x$ (when other stock variables change), and a linear increase in $\bar{k}_x$. The present lack of appropriate data suggests a need to test these assumptions under field conditions. Because Pacific salmon usually experience high rates of mortality, particularly during juvenile migrations into coastal waters, it is reasonable to assume a very high frequency of $k = 0$ (i.e. a large value for the y-intercept), an average value ($\bar{k}$) between 1 and 4, and a very low frequency of large values of k. The conditions can be met by a Poisson distribution, a negative exponential distribution, or even a bimodal or multimodal curve described by a polynomial function. A constant value for each $\bar{k}_x$ is a simplifying assumption that ignores complicating factors, for example, density dependence. Evidence for increases in the fecundity of lake whitefish (*Coregonus clupeaformis*), lake trout (*Salvelinus namaycush*), and some British Columbia coho salmon (*Oncorhynchus kisutch*) stocks after heavy exploitation suggests that fecundity is influenced by stock abundance in these fishes (Beacham 1982). Because fecundity is a major component of fitness (k = fecundity × survival), $\bar{k}_x$ values may also change as stock abundance changes. The assumption of a linear increase in $\bar{k}_x$ as age (x) increases is based upon the linear increase in fecundity as age increases in Pacific salmon. This results from a linear relationship between fecundity and length

where length increases as age increases (Beacham 1982). However, a linear increase in $\bar{k}_x$ also requires that the average survival of gametes per parent at each age is constant or nearly so.

In this example, only one set of parameter values (i.e. slope and intercept) was used for linear increases in $\bar{k}_x$ in order to simulate age-specific differences in fitness. But if there is a greater difference among $\bar{k}_x$ values (for $x = 2-5$) in the chum stock, then there would be a greater difference between the elevations of the lines for ages 2−5 vs. ages 2−3 or ages 3−4 (Fig. 2 and 4). Thus, the sensitivity of $N_{e(v)}$ to age structure in natural stocks may be very different from that shown here for fictitious stocks. Application of the model to fisheries management would require parameter estimation for all age classes in the managed stocks.

Discussion

The conceptual model we propose is an extension of the stock concept intended to insure the long-term reproductive fitness of stocks. An important feature of the model is that it is a phenotypic model.

The combinations and permutations of genetic and environmental processes that regulate fitness approach infinity. A priori, understanding the frequencies of specific genes in managed populations cannot enable us to manage effectively the genetic health of the populations because the sequence of environmental circumstances to be encountered through the life history of the resource cannot be predicted before the fact. Additionally, individual phenotypic responses can only be predicted within broad ranges, even in cases where the individual genotypes are known, because of pleiotropic and other interactive effects (Falconer 1981; Gjedrem 1983; Kincaid 1983; Kirpichnikov 1981; Birnbaum 1972; Lewontin 1982). It is often overlooked that there may be substantial phenotypic variation in isogenetic populations.

It follows from the preceding considerations that it is desirable to model fitness in phenotypic space. A phenotypic fitness model provides substantial flexibility in adapting the model to age, growth, fecundity, and other aspects of the life history patterns of the species of concern. Most importantly, it offers the advantage of enabling one to deal quantitatively with population fitness using only estimates of population variables routinely recorded or capable of being recorded in fisheries survey work, thus avoiding the necessity of making direct estimates of other genetic properties of populations.

We regard the latter point to be of paramount importance. Whereas classical population genetics focuses upon the genetic structure of populations, natural resource management is more concerned with the phenotypic expression of the genetic information in a variable and unpredictable environment. Therefore, although the frequencies of various alleles in populations may be useful in identifying stocks as management units, such information is of limited utility in making decisions about how the stocks should be managed. In attempting to relate gene frequencies to stock management, one must assume that the gene frequencies are correlated with reproductive fitness irrespective of whether the frequencies are determined from electrophoretic evaluation of protein polymorphisms, meristic traits, or any other methodology. The validity of this assumption is tenuous at best, for in addition to the technical questions associated with the methods of analysis, it is further assumed that the observed variability in a limited number of structural genes reflects the variance of population fitness. If this assumption is valid, then (1) the loci observed are the major ones determining fitness and (2) the loci act independently in their contribution to fitness. The likelihood that a structural gene with these properties exists is extremely remote.

It would be desirable to derive a priori probability distributions of fitness for use in management models. For most species, this is not possible at the present time because the available life history information is inadequate for this purpose. For the present, we have suggested using an empirical baseline for the probability distribution of fitness that is "at a value consistent with management goals." Thus, the possibility still exists to modify inadvertently population structure unless careful attention is paid to the appropriate selection of reference generations for the baseline.

Application of a fitness model requires determining when the probability distribution of fitness is at an acceptable value. In answering this question it must first be recognized that the model requires biological as opposed to political acceptability. Fitness cannot be maintained in the face of politically mandated overfishing.

The selection of appropriate reference generations demands careful attention to biological detail. In selecting this biological baseline, it is essential to give consideration to life history patterns, short- and long-term population trends, and the influence of harvest methods on patterns of stock abundance.

For species that show year-to-year variation in abundance or sex ratio, the reference probability distribution of fitness should be determined over several breeding seasons. If only a single breeding season is used and the population is either increasing or decreasing in abundance, the probability distribution of fitness may deviate from that of a stable population. Average fitness will decrease in breeding seasons when population numbers decrease, and will increase in seasons when the population numbers increase. A similar response will be seen in the variance effective number calculated from the equations we have presented. For example, the variance effective number decreased when a fictitious stock was in decline and increased when the stock was growing (Fig. 3).

Many species appear to undergo long-term cyclic changes in abundance. In these cases it is important to understand the position of the reference generations relative to this cycle. Again, the probability distribution of fitness will vary depending upon whether the population is at the low point of the cycle, at maximum abundance, or at some intermediate level of increase or decrease. The variance effective number will vary in a corresponding manner (e.g. see results for Fig. 5).

In summary, trends in population abundance must be evaluated when selecting reference breeding seasons for the empirical baseline. It is useful to remember that in a stable population (i.e. one that is maintaining constant numbers from one generation to another) the average fitness is one successful progeny or two successful gametes per parent. Additionally, a population which is experiencing a decline in abundance probably needs to maintain a broader variance of fitness than one which is increasing in abundance. In all cases it is essential to consider reproductive strategies, life history patterns, and patterns of environmental change including climatic factors in the selection of appropriate reference generations.

In some instances it may be possible to select reference generations from an era in which stocks are not exploited. A more typical situation is that fishery statistics provide the data for estimating population trends. In this case it is necessary to

consider the influence of harvest methods on patterns of stock abundance. For example, consider a stock of marine fish composed of individuals representing a large number of age classes. If fishing selectively extracts the older age classes (presumably the larger fish), the average age of reproduction is reduced. If there is a genetic component to the age class structure, this is tantamount to modifying the probability distribution of fitness for this stock. There is a corresponding modification of the output from the equations we have presented for the model. Figures 2 and 4 illustrate a decrease in the variance effective number when older age classes are deleted from a fictitious chum salmon stock.

In developing the example of functional relationships for the model, we have relied heavily upon the contributions of Kimura and Crow (1963) and Crow and Kimura (1970). Other workers (e.g. Choy and Weir 1978; Emigh and Pollak 1970; Felsentein 1971; Hill 1979; Nei and Imaizumi 1966; Robertson 1961) have contributed alternate derivations for effective population numbers which also would be suitable as functional relationships in application of the conceptual model.

We have elected to use the equations of Kimura and Crow for a number of reasons. Because they have appeared in the literature for some 20 yr, they are familiar to the community of population biologists. In spite of their simplifying assumptions, they provide substantial flexibility in adapting the model to the life history patterns of the species of concern. Furthermore, they require inputs that are estimates of population variables routinely recorded or capable of being recorded in fisheries survey work.

The present forms of the functional relationships provide a working hypothesis for assessing the possible impacts of exploitation and management on the genetic health of salmon stocks, or for making allocation decisions consistent with the goal of maintaining the variance effective number at an adequate level. Their use in a worked example led to conclusions about the genetic impact of changes in age structure, stock abundance, and immigration that follow the same pattern as in our previous application of a fitness model to Pacific salmon management. However, caution is urged in their application to the development of any genetic stock management owing to certain assumptions and unknowns. The principal assumption is that the relationships among the population variables in the classical formula for $N_{e(v)}$ (Crow and Kimura 1970) are appropriate for the translation of the conceptual model into quantitative terms. Although this formula was selected after careful consideration of other quantitative expressions of population genetics, it may be possible to develop more relevant relationships (among the various population variables) from first principles.

A second assumption of management concern is that environmental conditions in past and present breeding seasons are sufficiently similar to warrant equating $N_{e(v)}$ to $N'_{e(v)}$ in expression (3). When environmental conditions fluctuate around a mean value, this assumption may be tolerable. However, expression (3) will need modification if the historical record shows a unidirectional change in the value of important physical parameters (e.g. water temperature). It may be possible to add coefficients to correct the values of population variables on either side of equality (3), but only if enough is known about the impact of the physical parameters on these variables. Thus, the selection of reference breeding seasons will be greatly complicated under the conditions of unidirectional environmental trends.

Management must recognize also the lack of density-dependent behavior in the existing functional relationships for the model. This may not be a serious limitation if the functional relationships are eventually incorporated into a population dynamics model that exhibits density dependence (e.g. the Ricker stock–recruit function for Pacific salmon). Additionally, it may be possible to include interaction terms for age-specific mean fitnesses or variances of fitness (e.g. covariance terms) into the existing functional relationships. This will require some estimation from empirical evidence for such age-specific interactions.

Practicable application of the quantitative model to stock management is constrained by the estimations of $s^2_{k,x}$ values in equation (13) for the calculations of V_k and V'_k in equation (16). If the estimations of $s^2_{k,x}$ values require field sampling, there may be considerable cost and time delay involved in the application of equation (16) to the management of a given stock. The magnitude of this problem will be influenced by the life history of the species. Additionally, data gaps in the historical record may preclude computation of the necessary variables in equation (16) for some stocks.

Data constraints may be circumvented by developing indirect estimates of the population variables required for equation (16). A simple example is the calculation of $s^2_{k,x}$ from estimates of age-specific fecundity and survival by

$$s^2_{k,x} = f(\bar{k}_x) = f\left[(\text{fecundity}_x)(\text{survival}_x)\right]$$

where fecundity could be estimated as a function of length or weight at age (Pitcher and Hart 1982). However, indirect estimates will contribute error to the output of equation (16) and will increase the uncertainty involved in the application of equation (16) to stock management. Consideration must be given to such uncertainty if conservative management is desired.

The functional relationships we presented are applicable to the long-term perpetuation of a particular fishery resource. The conceptual model and the functional relationships together provide a rational approach to considering the long-term genetic implications of fisheries exploitation and management.

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Vertical Distribution and Habitat Partitioning Between Different Size Classes of Vendace, *Coregonus albula*, in Thermally Stratified Lakes

Stellan F. Hamrin

Fish Ecology Research Group, Institute of Limnology, Department of Ecology, University of Lund, P.O. Box 65, S-22100 Lund, Sweden

Hamrin, S. F. 1986. Vertical distribution and habitat partitioning between different size classes of vendace, *Coregonus albula*, in thermally stratified lakes. Can. J. Fish. Aquat. Sci. 43: 1617–1625.

Diel vertical distribution of juvenile and adult vendace, *Coregonus albula*, was studied acoustically and by gill netting during summer in thermally stratified lakes in southern Sweden. Daytime distribution of adult vendace was restricted to hypolimnion (minimum 5°C). During nocturnal vertical migrations, adults moved into the metalimnion, but temperatures greater than 18°C were avoided. In contrast, juvenile vendace were often found in the warmer and more shallow water of the metalimnion during the day. At night, the juveniles experienced a temperature change of 10° as they migrated into the epilimnion. All age classes had a nocturnal/crepescular diel rhythm. The diel vertical distribution of vendace is interpreted as a response to temperature stratification and to diel changes in light intensities. The different responses of the age classes are influenced by ontogenetic changes in temperature and light preferences as well as by size-related intraspecific interactions.

On a étudié à l'aide d'appareils acoustiques et de filets maillants la distribution verticale nycthémérale de corégones blancs (*Coregonus albula*) jeunes et adultes pendant l'été dans des lacs où il y avait une stratification thermique, dans le sud de la Suède. La distribution des corégones blancs adultes pendant le jour était limitée à l'hypolimnion (minimum de 5°C). Au cours des migrations verticales pendant la nuit, les adultes se sont rendus dans le métalimnion, mais ils évitaient les températures supérieures à 18°C. Par contre, on trouvait souvent de jeunes corégones blancs dans les eaux plus chaudes et moins profondes du métalimnion pendant la journée. La nuit, les jeunes subissaient un changement de température de 10°C lorsqu'ils se rendaient dans l'épilimnion. Toutes les classes d'âge avait un rythme nycthéméral nocturne crépuscule. La distribution verticale nycthémérale du corégone blanc est interprétée comme une réaction à la stratification thermique et aux changements nycthéméraux dans l'intensité de la lumière. Les réactions différentes des classes d'âge sont influencées par des changements ontogéniques dans les préférences en matière de température et de lumière de même que par les interactions intraspécifiques liées à la taille.

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In pelagic, zooplanktivorous fishes such as vendace, *Coregonus albula* (Hamrin 1983, 1986), oscillations in population size have often been reported (Huitfeldt-Kaas 1927; Järvi 1942; Nordquist 1944; Svärdson 1956; Aass 1972). Several explanations have been advanced for the oscillations (e.g. Järvi 1942; Svärdson 1956; Birman 1975), although those based on climatic conditions and predation have been rejected (Svärdson 1956; Aass 1972). Intraspecific competition has been suggested as an explanation for the oscillations (Aass 1972; Hamrin 1979). One such hypothesis suggests that intraspecific food competition during the period of gonad development between young-of-the-year (YOY) and adult (yearling) vendace produces a 2-yr oscillation in year class strength (Hamrin and Persson 1986). Habitat segregation between age classes of vendace might therefore be expected as an adaptation or as a response to intense competition for food (Schoener 1974; Brandt 1980). As food resources are depleted over the summer, intraspecific competition is intense and growth rates are greatly reduced from July through October (Hamrin and Persson 1986). For coldwater species like vendace (Northcote and Rundberg 1970; Hamrin 1986), opportunities for segregation is enhanced by vertical thermal stratification of lakes.

The purpose of this study is to evaluate the hypothesis that habitat segregation between different size classes of vendace occurs during summer and may reduce intraspecific competition.

Materials and Methods

Study Areas

The six studied lakes (size from 0.4 to 181 km^2) are located at altitudes between 6 and 277 m in southern Sweden (Table 1). They are oligotrophic except for mesotrophic Lake Ivösjön and with transparencies varying from 1.6 to 11.2 m. They thermally stratify during summer.

Vendace spawn in early November, eggs hatch at ice-break, generally in April, and the fry reach a total length of approximately 10 cm by autumn. They spawn in their second (Lake Bolmen) or third autumn at a total length of 13–16 cm. Maximum length varied between 18 and 25 cm in the different lakes.

TABLE 1. Physical and chemical data on the six studied lakes in southern Sweden (S. F. Hamrin, unpubl.). Samples taken April–October.

Lake	Coordinates	Altitude (m)	Area (km^2)	Max. depth (m)	Conductivity (μS)	pH	Total P (μg/L)	Secchi disc transp. (m)
Bolmen, 1970–74, n = 20	13°45′E 56°51′N	141	181	37	57–70	6.4–7.0	6–16	4.0–5.9
Ivösjön, 1974, n = 12	13°55′E 56°7′N	6	54	50	130–156	7.4–8.1	9–20	5.0–7.8
Fegen, 1974, n = 30	13°9′E 57°12′N	132	24	36	53–74	5.6–6.9	8–17	3.6–6.1
Skärlen, 1972, n = 2	14°53′E 57°10′N	212	3	27	46	6.5	3	10.3–11.2
Skärsjön, 1977, n = 1	14°30′E 57°45′N	277	0.9	35	63	7.3	17	7.0
Gyltigesjön, 1972, n = 2	13°12′E 56°46′N	65	0.4	18	56–65	6.1–6.3	14–15	1.6–2.0

TABLE 2. Percent composition of gillnet catches in the epi-, meta-, and hypolimnion (E, M, and H, respectively) in each lake in July. The numbers give percentage of the total catch (n) of fish (>90 mm) on three dates. The abundance of smelt is underestimated (see the text). Scientific names are given in the text.

Lake	Vendace	Whitefishes	Smelt	European perch	Roach	Other species	n
Bolmen, 1972							
E (1–4 m)	6	0		89	2	3	352
M (10–13 m)	25	3		62	4	6	142
H (21–24 m)	80	12		5	0	3	422
Ivösjön, 1973							
E (1–15 m)	30		0	52	10	8	50
M (25–28 m)	63		14	23	0	0	51
H (40–43 m)	95		2	1	0	2	422
Skärlen, 1974							
E (1–4 m)	2	0		5	93	0	86
M (8–11 m)	82	0		8	8	2	96
H (15–18 m)	75	22		0	0	3	38
Skärsjön, 1977							
E (1–4 m)	20	0		0	80	0	20
M (5–12 m)	100	0		0	0	0	9
H (13–24 m)	100	0		0	0	0	32
Gyltigesjön, 1974							
E (1–4 m)	0			7	93	0	976
M (8–11 m)	29			29	42	0	7
H (15–18 m)	100			0	0	0	19

Sampling

Fish species composition was estimated with pelagic gill nets set at three depths in each lake (gill net area per depth and date was 720 m^2; monofilament nylon, mesh sizes 20, 30, 33, 44, 50, 60, 76, and 100 mm stretch). Vertical distribution of adult vendace was studied with other gill nets (area 90 m^2; monofilament (Lake Bolmen) or twined nylon, mesh sizes 20, 30, and 33 mm). All nets were set at three dates at 09:00 standard time and pulled 24 h later.

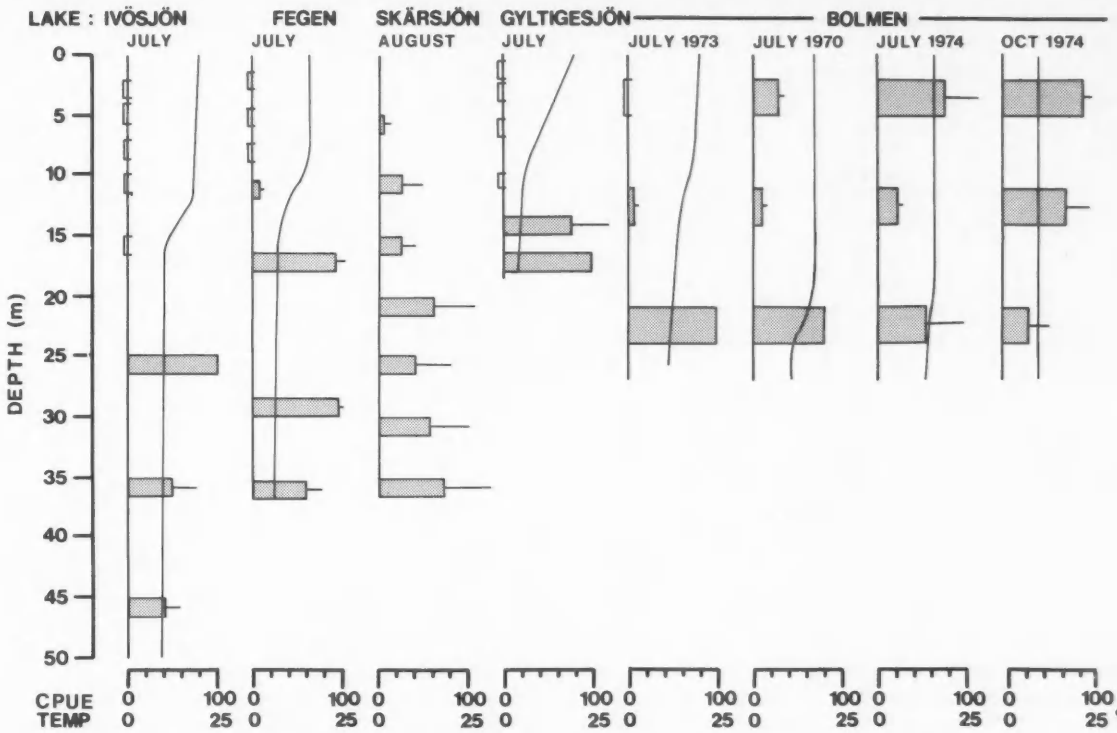


FIG. 1. Mean CPUE (n = 3) and SE (horizontal line when >5%) of adult vendace at different depths in Lakes Ivösjön (total number of vendace caught = 28), Fegen (189), Skärsjön (77), Gyltigesjön (9), and Bolmen (870, 805, 146, and 543, respectively). Square indicate catches of zero vendace. Temperature at different depths is given by the solid vertical line. CPUE is expressed as percentage of the highest value for each lake.

Diel rhythm of adult vendace was studied on seven dates during the year, with a vertically placed gill net (area 180 m^2; monofilament nylon, mesh sizes 22, 33, and 38 mm) set at 09:00 and pulled 3–6 h later depending on time of day and year. Catch per unit effort (CPUE) was defined as number of fish caught per gang of nets and time period after correction for the length selectivity of the mesh sizes (Hamrin 1979).

Diel vertical movements of juvenile and adult vendace were also studied by echo sounding (Atlas Echolot Mönograph 58, 12 V, 80 kHz). Profiles of temperature and oxygen were estimated with a thermistor and a lead–silver probe (EIL-instruments) and recorded for every 2 m of depth during each fishing period (1–4 d). The values were checked against mercury thermometer readings (surface water) and Winkler measurements (surface and bottom water). Light penetration was measured with a quantum/lux meter (LI-185, Lambda Inst. Co.) with an underwater silicon photodiod lux sensor.

Results

Fish Species Composition

Based on gillnet catches, vendace was the most abundant coldwater planktivorous fish in all six lakes (Table 2). In Lake Ivösjön, smelt (*Osmerus eperlanus*) was the most abundant species in samples collected by explosives (S. F. Hamrin, unpubl.). Individuals belonging to the three youngest age classes were too small to be caught in the smallest mesh size we used (20 mm stretch). Other common species in all lakes were European perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), and northern pike (*Esox lucius*). Among planktivorous species, whitefishes (*Coregonus* spp.) occurred in moderate numbers in all lakes except Lake Ivösjön and Lake Gyltigesjön.

Habitat Utilization

Adult vendace concentrated in the hypolimnon whenever distinct thermal stratification existed (Fig. 1). In Lakes Ivösjön, Fegen, Skärsjön, and Gyltigesjön, catches were significantly greater in the hypolimnion than in the epi- plus metalimnion (Mann-Whitney T-test, $p < 0.05$). A few fish were caught in the epilimnion if water temperature did not exceed 18°C. In Lake Bolmen, thermal stratification varied among years (Fig. 1). Adult vendace were significantly more abundant (Mann-Whitney T-test, $p < 0.05$) in the hypolimnion compared with the meta- plus epilimnion, when the hypolimnion volume was large and its temperature low (e.g. 1973, temperature 10°C). When the hypolimnion was small and warmer (Lake Bolmen, July 1970 and 1974), the relation between abundance and thermal strata was not significant ($p > 0.1$). Also, adult vendace were not concentrated in deep water during autumn mixes (e.g. 1974). The oxygen content in the lower part of the hypolimnion exceeded 4 mg/L in all lakes during our sampling.

The CPUE of adult vendace in both southern and northern parts of Lake Bolmen decreased nonlinearly from 10 to 18°C

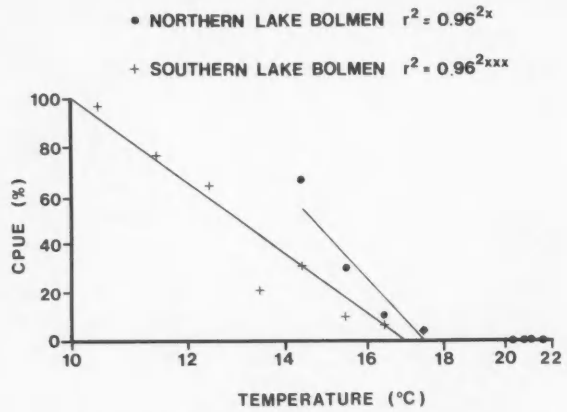


FIG. 2. CPUE of adult vendace at different temperatures (log values) in July 1970–72 in northern and southern Lake Bolmen. x and xxx indicate r values significant at the 0.95 and 0.999 significance levels. CPUE is expressed as percentage of the highest value. The regressions lines are based on values <18°C.

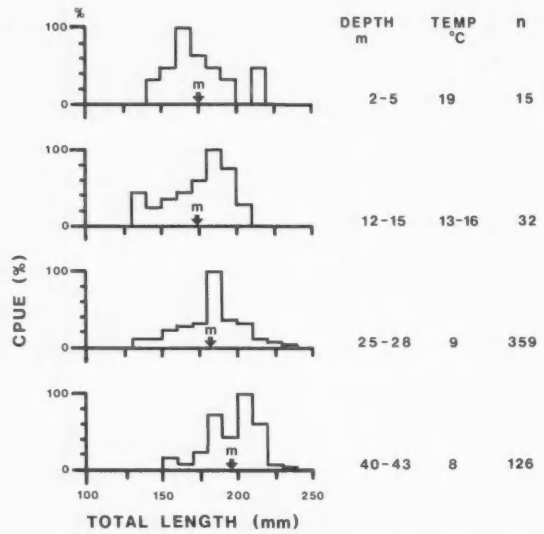


FIG. 3. CPUE of different size classes of adult vendace at different depths in central Lake Ivösjön (July 14–17, 1973). n is the number of vendace caught at each depth interval and m is the mean length at each depth (SD = 22.3 for all depths). CPUE is expressed as percentage of the modal value for each depth.

($r^2 = 0.96$, $p < 0.05$ for both parts). No fish were caught in water with temperatures above 18°C (Fig. 2). When testing the data from all five lakes (Lake Bolmen, 1973), temperature and abundance of adult vendace were significantly related (Friedman two-way ANOVA for randomized blocks; three temperature classes; $\chi^2 = 6.2$, $p < 0.05$).

In the homothermic hypolimnion of Lake Ivösjön, adult vendace were more abundant in the upper and most illuminated part (light penetration 0.0001%; maximum 0.05 lx), even though temperatures were the same.

The length distribution of adult vendace changed with depth in the main basin of Lake Ivösjön (Fig. 3). The mean length

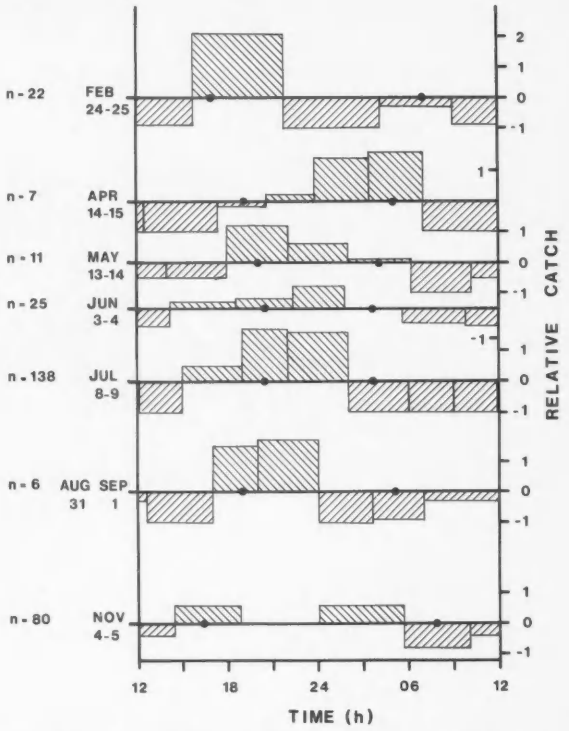


FIG. 4. Relative catch of adult vendace in northern Lake Ivösjön in 1976 expressed as $(a_1 - a_m)/a_m$ where a_1 = number of vendace caught per hour and a_m = mean number caught per hour during 24 h. n is the total number of vendace caught per 24 h. Times of sunrise and sunset are indicated for each depth (●).

was significantly lower in the hypolimnion (9°C) than in the meta- plus epilimnion (13–19°C; t-test, $p < 0.05$).

Diel Activity and Migrations

Throughout the year the catches of adult vendace were highest from dusk through dawn (Fig. 4; $\chi^2 = 32.4$, $p < 0.001$) with highest values during the first part of the period ($\chi^2 = 15.95$, $p < 0.01$). Daytime catches (1 h after sunrise to 1 h before sunset) were low. During the whole 24-h period, the distribution of adult vendace was mainly restricted to the hypolimnion (Fig. 1).

Examination of echo sounding charts (80 kHz) showed marked differences between Lake Skärlen and Lake Gyltigesjön in 1974 (Fig. 5). In Lake Skärlen, only a few scattered targets concentrated around 5 and 15 m were visible at night. At dawn the lower targets move towards the bottom (light penetration 0.5%; maximum 200 lx) and leave the pelagic zone. In Lake Gyltigesjön, a distinct band of overlapping targets moves from the bottom during day toward the surface at night.

The lower group of targets in Lake Skärlen should mainly be adult vendace because (1) vendace constituted the major part of the hypolimnic fish fauna (Table 2), (2) adult vendace were restricted to the hypolimnion (unpubl. gillnetting results; cf. also Fig. 1), and (3) vendace fry were very rare during 1974 as indicated by gill netting in the autumn (S. F. Hamrin, unpubl.).

The targets in Lake Gyltigesjön, however, should be

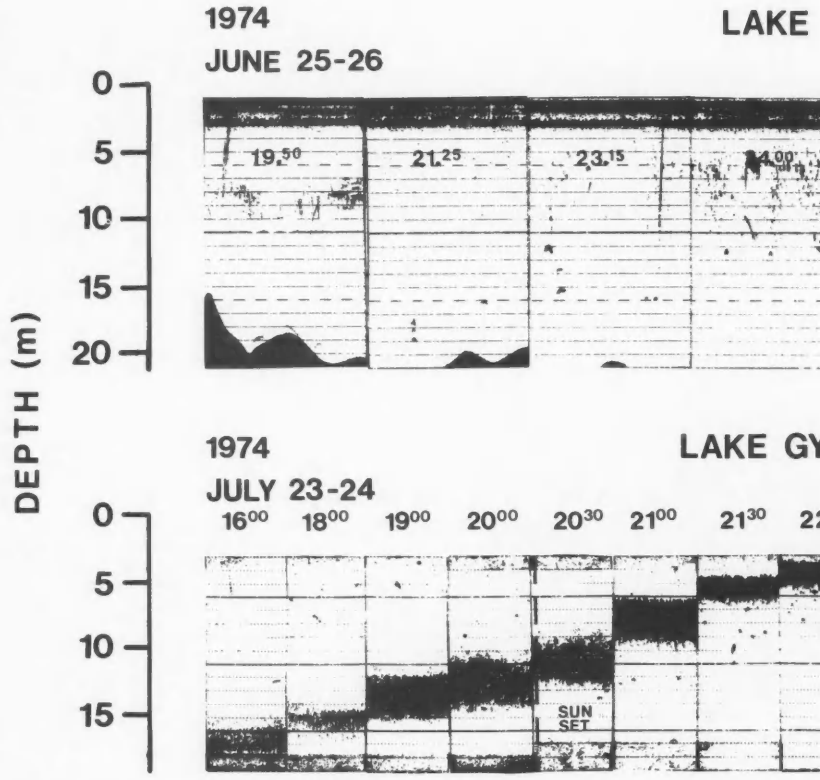
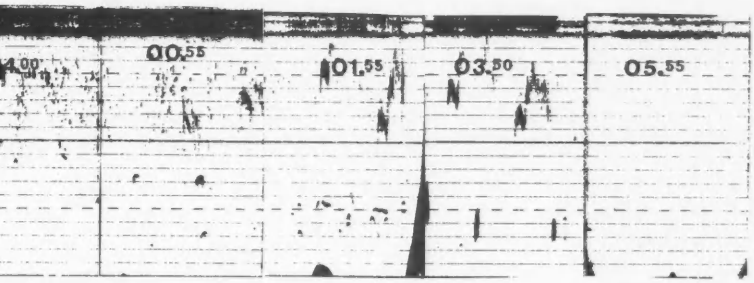
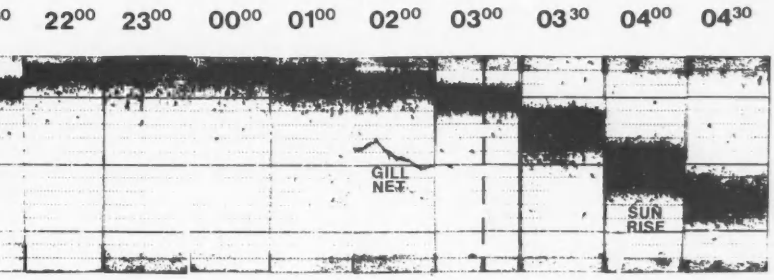


FIG. 5. Echo sounding charts from Lake Skärlen (1974) and Gyltigesjön (1974) showing vertical mi

AKE SKÄRLEN



E GYLTIGESJÖN



rtical migrations of adult and YOY vendace, respectively. See the text for further explanation.

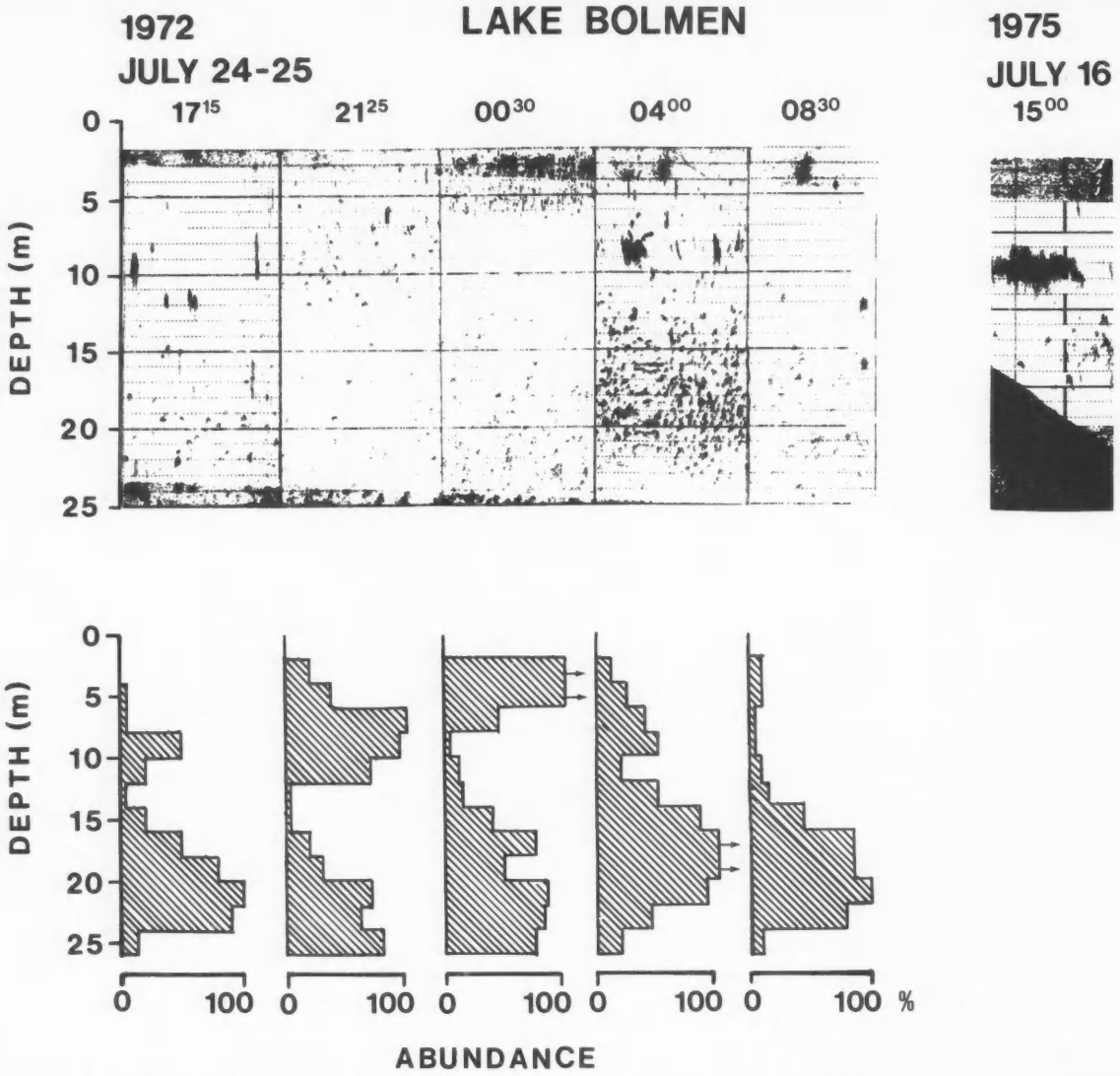


FIG. 6. Echo sounding charts from Lake Bolmen in 1972 and 1975 (far right) during years with weak and strong year classes, respectively, showing the vertical distribution of YOY (above) and adult (below) vendace. Number of fish echos is given in the lower figure. See the text for futher explanation.

vendace fry (no other fish species occur in the hypolimnion; Table 2). This year a large cohort of vendace was formed in the lake, the first large one in 5 yr (verified by gill netting in 1975; cf. Hamrin 1986), while at the same time the adult population was small (approximately 500 ind./km^2; Hamrin 1979). The band is not likely produced by microzooplankton owing to the sonar frequency (McNaught 1968; Yudanov 1971; cf. also Hamrin 1979 for weak cloud-shaped nonmigrating echoes of *Daphnia longispina* from Lake Gyltigesjön in 1972). The band is not *Chaoborus* either (the only macrozooplankton existing in the lake) because the echoes seen in 1974 did not appear during 1972, 1973, and 1975, years with very weak vendace populations. Thus, these data suggest that vendace fry have a marked vertical migration. The smaller targets in Lake Skärlen at 5 m may also have been vendace fry, but were more likely produced by horizontally moving epilimnic species such as roach or perch (cf. Northcote and Rundberg 1970).

In Lake Bolmen the occurrence during the day of two layers of fish in the meta- and hypolimnion, respectively (Fig. 6), was detected using sonar. The upper layer was more pronounced during 1975 when YOY abundance was high (cf. Hamrin and Persson 1986). Daytime trawling catches (mouth diameter 5 m, cod end mesh size 10 mm stretched) showed a dominance of YOY over adults in the metalimnion (18 to 2; mean value for three hauls) and the reverse in the hypolimnion (3 to 31). During night the upper layer appeared to migrate to the epilimnion (from 15 to 19°C), while the lower layer stayed within the hypo- and metalimnion (temperature range 13–15°C). The

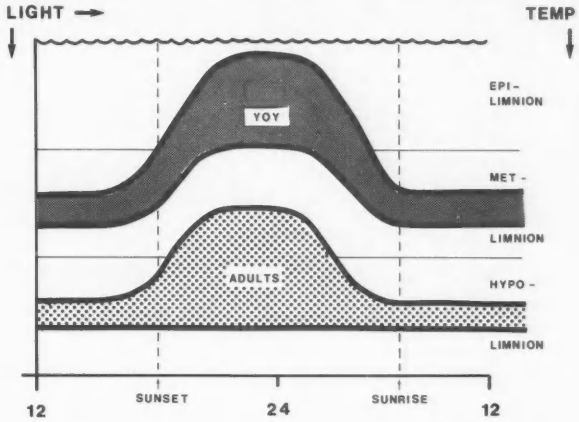


FIG. 7. Schematic view of the diel vertical migration of YOY and adult vendace with the most important regulating factors indicated. See the text for further explanation.

hypolimnic distribution of adult vendace is corraborated by results from gillnet catches (Fig. 1) and by the increase of vendace mean size with depth in Lake Bolmen (Hamrin 1979) and Lake Ivösjön (Fig. 3).

Discussion

Differences in light intensity and temperature could bias the number of fish collected at each depth by affecting net avoidance behavior and locomotor activity (Hamley 1975). Vendace probably do not show net avoidance because (1) trout (another salmonid) do not to avoid monofilament gill nets in high transparency lakes (Steinberg 1961), (2) the results from gill netting are consistent with those obtained using sonar, and (3) the catches were high at dusk but low at dawn even though light intensities were similar. As temperature increases, so does swimming speed (Webb 1975). However, water strata with higher temperatures were avoided by adult vendace, so the temperature effect on CPUE is of minor importance.

The vertical distribution of adult vendace was related to temperature, with no fish found in waters above 18°C (Fig. 2; see also Walczak 1953; Northcote and Rundberg 1970) and with the largest catches from the coldest waters. Thus, adult vendace would occupy the meta- and hypolimnion during summer stratification, while the epilimnion is unsuitable.

The profundal daytime distribution of adult vendace in the highly transparent Lake Skärlen might be a light avoidance response similar to that found for perch (Rudstam and Magnuson (1985)). In lakes with lower transparencies, adult vendace remained pelagic. In the homothermal hypolimnion of Lake Ivösjön, adult vendace occupied the upper part of the hypolimnion. Vendace were thus found in water strata with maximum light intensities between 200 and 0.05 lx.

The nighttime vertical distribution of adult vendace was unaffected by high light intensities. In spite of this, both gill netting and sonar charts indicated that adult vendace remained in the hypo- or metalimnion at night (Fig. 1 and 5 and Lieder 1962; Dembinski 1971). YOY vendace, however, migrated to the epilimnion experiencing a broader range of temperatures. In Lake Gyltigesjön, the temperature range was approximately 10°C, equal to the maximum temperature range found for both warm- and coldwater fishes (Magnuson et al. 1979). The shallow depth distribution of YOY vendace is consistent with other studies of vendace (Lieder 1962; Northcote and Rundberg 1970; Dembinski 1971) as well as of other planktivorous fish (Brandt 1980; Crowder et al. 1981; Rudstam and Magnuson 1985).

I suggest that the diel vertical distribution of vendace is a response to temperature and light. Depending on the environment, these could act both as controlling and directive factors (sensu Fry 1971). When predation is high and interspecific competition low, the diel vertical distribution of vendace in thermally stratified lakes should be as follows (Fig. 7).

Daytime distribution of vendace is restricted to the meta- and hypolimnion. YOY vendace occur in warmer water with higher light intensities than adults. The preferred water strata is basically a response to temperature, which however might be modified by high light intensities. Locomotor and feeding activity increase at dusk, reach a peak during early night, and decrease before dawn. YOY vendace undergo vertical migrations, which, depending on the temperature, might include movements to the epilimnion. Adult vendace remain in cold water but could expand their temperature zone a few degrees. The habitat segregation between YOY and adults is maintained during this period.

Vertical migrations of fish have been explained as responses to temperature, light, and food availability (Brett 1971; Eggers 1978; Brandt 1980; Janssen and Brandt 1980; Crowder and Magnuson 1983). Their relative importance should, however, be dependent on the size and species of the fish and on environmental factors.

The lethal temperature of adult vendace is 23–24°C (Eichler 1940; EIFAC 1969). The upper avoidance temperature of 18°C in the south Swedish lakes (see also Walczak 1953; Northcote and Rundberg 1970) might therefore be a direct effect of temperature, but it could also be influenced by low oxygen concentration (cf. Fry 1971; Webb 1978).

Below 18°C, temperature could act as a directing factor by which vendace optimize their metabolism (behavioral thermoregulation). Because of ontogenetic changes in temperature preference (McCauley and Huggins 1979), larger fish should occur at greater depths than smaller fish in thermally stratified lakes. This vertical segregation might be reinforced by asymmetrical food competition (Hamrin and Persson 1986), selecting against larger individuals. By behavioral thermoregulation, adults may lower their temperature preference more than YOY (Brett 1971). This would explain the occurrence of adult vendace in 5°C water strata, which is much less than the preferred temperature of other salmonids fed ad libitum rations (12°C; Rudstam and Magnuson 1985).

Light was obviously a directive cue for the diel rhythm of vendace by initiating their activity at dusk and reducing it at dawn. This agrees with earlier studies of vendace (Northcote and Rundberg 1970) as well as other pelagic fishes (Ali 1959; Blaxter 1974).

Light also influences the vertical distribution. Under homothermal conditions, vendace move to the epilimnion as light intensity decreases (Enderlein 1981). In a temperature gradient this behavior was unaffected for YOY vendace, while adults remained in the meta- or hypolimnion during the night. Ontogenetic changes in light perception (Breck and Gitter 1983) could partly explain differences in the light response. In stratified lakes, however, the lower preferred temperature of adult vendace is probably why adults do not remain at their preferred

light intensity.

The daytime light avoidance reaction of vendace could be due both to the light intensity per se and to the predation risk (cf. Eggers 1978; Magnuson et al. 1979; Brandt et al. 1980). The high CPUE of adult vendace in Lake Bolmen during autumn, which indicates a more or less permanent occupation of shallow water strata, does not support the first alternative. Vendace are small fish with high yearly mortality rates (80–100%; Hamrin 1979, 1986). In pelagic habitats, darkness is the only antipredator shelter available, and the adaptive value of reducing predation risk must be high.

The habitat segregation between YOY and adult vendace persisted throughout the 24-h period. The food overlap between size classes could then temporarily be reduced (Hamrin and Persson 1986) when the lakes are thermally stratified. It is, however, unlikely that the vertical migrations of vendace are influenced by prey availability (Janssen and Brandt 1980). Studies of copepods and cladocerans in the central part of Lake Ivösjön did not indicate any diel vertical migrations which could explain the large migrations performed by both vendace and smelt in this lake (S. F. Hamrin, unpubl.). This assumption, however, needs further verification.

Habitat shifts might occur due to interspecific interactions (Werner et al. 1977, 1983; Crowder and Magnuson 1982). In most Fennoscandian lakes, vendace is the dominate planktivore, although it occurs sympatrically with smelt in a few lakes (Svärdson 1976). In one such lake, Lake Mälaren, adult vendace sometimes occur in the epilimnion in large numbers at dusk (Northcote and Rundberg 1970). This has also been reported from peripheral basins of Lake Ivösjön (Hamrin 1979). In these, interspecific competition with smelt might be more intense than in the central part, where adults remain in the meta- and hypolimnion (Fig. 1). In lakes without smelt, adult vendace are not found in the epilimnion. Interactions with rainbow smelt (*Osmerus mordax*) have been suggested as the reason for the habitat shift in YOY ciscoe in one Wisconsin lake (Rudstam and Magnuson 1985).

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Transmission of *Diphyllobothrium ditremum* to Arctic Char (*Salvelinus alpinus*) in Two Subarctic Quebec lakes

Michel Bérubé and Mark A. Curtis

Institute of Parasitology, Macdonald College, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Que. H9X 1C0

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Diphyllobothrium ditremum (Cestoda: Pseudophyllidea) transmission to arctic char (*Salvelinus alpinus*) was investigated at two small (<10 ha) lakes in northern Quebec, where the char populations exhibit a marked difference in plerocercoid prevalence (78 vs. 18%) and abundance (27.7 vs. 1.2 plerocercoids/fish). The highly infected char population was characterized by slow growth and poor feeding conditions. Feeding behavior of char in the two lakes differed principally in the fall, when consumption of benthos significantly decreased in the highly infected population as char fed more on copepods or sticklebacks (*Gasterosteus aculeatus*). Presence of these two food items in char stomachs was associated with high abundance of *D. ditremum*. The availability of alternative foods, such as larval insects and amphipods, appears to prevent char from acquiring heavy infections of *D. ditremum*, even in circumstances where all potential hosts are present.

L'infestation de l'omble chevalier (*Salvelinus alpinus*) par le *Diphyllobothrium ditremum* (Cestode : pseudophyllidés) a été étudiée dans deux petits lacs (<10 ha) du nord du Québec où les populations d'ombles présentent des différences marquées touchant l'incidence (78 et 18 %) et l'abondance (27,7 et 1,2 plérocercoïdés/poisson) de l'infestation par plérocercoïdés. La population d'omble la plus infestée se caractérisait par une croissance lente et de mauvaises conditions d'alimentation. Le comportement alimentaire des ombles des deux lacs différait surtout en automne, lorsque la consommation d'organismes benthiques diminuait de façon appréciable chez la population fortement infestée, les ombles consommant plus de copépodes et d'épinoches (*Gasterosteus aculeatus*). La présence de ces deux types d'organismes dans l'estomac des ombles était liée à une forte abondance de *D. ditremum*. La disponibilité d'autres aliments, comme des larves d'insectes et des amphipodes, semblait prémunir les ombles des fortes infestations par *D. ditremum*, même en présence de tous les hôtes possibles.

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Diphyllobothrium ditremum Creplin, 1825 (Cestoda: Pseudophyllidea) occurs as a plerocercoid in the viscera of arctic char (*Salvelinus alpinus*), usually encysted on the stomach and other internal organs of its host. In common with other *Diphyllobothrium* species, the wandering plerocercoids of *D. ditremum* cause internal hemorrhaging and form adhesions binding the viscera together (Hickey and Harris 1947; Vik 1957; Bylund 1972; Becker and Brunson 1967). Because of the objectionable appearance of such fish, char populations containing many noticably infected individuals are not often exploited by man (Vik 1973).

Most lake resident populations of arctic char bear mixed infections of *D. ditremum* and *D. dendriticum* Nitzsch, 1824. *Diphyllobothrium dendriticum* is larger and more mobile than *D. ditremum*, and is generally considered more pathogenic to the fish (Halvorsen 1970; Henricson 1977). Unlike *D. ditremum*, *D. dendriticum* is infective to man although its normal definitive hosts are gulls (von Bonsdorff 1977). In the present case, *D. ditremum* represents 98% of all the plerocercoids recovered. This provides an occasion to study the biology of *D. ditremum* separately, without the constraints imposed by the presence of an abundant congeneric form.

The life cycle of *D. ditremum* includes a procercoid stage in copepods, a plerocercoid stage in fish (primarily salmonids and sticklebacks), and an adult stage in piscivorous birds of the genera *Mergus*, *Gavia*, *Phalacrocorax*, and *Ardea* (Vik 1964; Halvorsen 1970). Eggs passed into the water with the bird feces hatch into ciliated coracidia and, upon ingestion by a suitable copepod, the life cycle is continued. Plerocercoids of *D. ditremum*, like other species within the genus, can be transmitted to piscivorous fish from their prey, and the parasite may then accumulate in individuals feeding extensively on infected forage fish (Curtis 1982). Consequently, fundamental differences between fish feeding behavior from lake to lake must be considered important determinants in parasite transmission.

The present paper compares *D. ditremum* acquisition patterns in two small lakes in northern Quebec, one with an arctic char population lightly infected and the other heavily infected. By comparing parasite abundance and char feeding behavior in the two lakes, it is possible to evaluate factors involved in the acquisition of *D. ditremum* under different habitat conditions.

Description of Study Site

Lac Kitturiak (57°49′N, 69°28′W) and Lac Milugiak (57°49′N, 69°27′W) are located about 70 km southwest

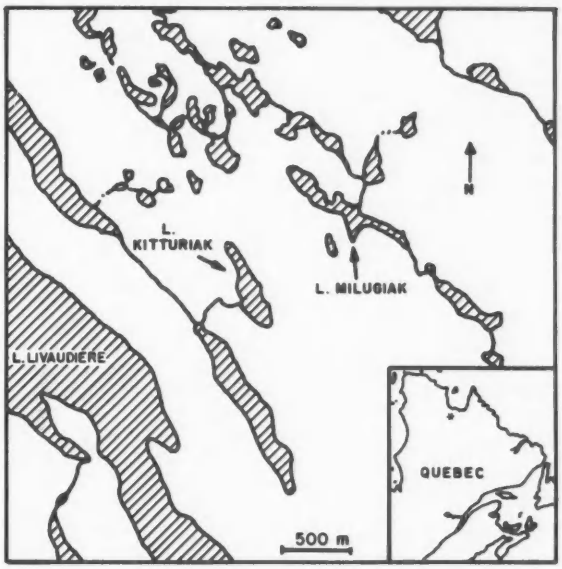

FIG. 1. Location of the sampling site, with relative positions of Lac Kitturiak and Lac Milugiak.

of Kuujjuak (Quebec), just north of the confluence of the Caniapiscau, Méleze, and Koksoak rivers. Situated at approximately 270 m above sea level, they contain only arctic char and three spined sticklebacks (*Gasterosteus aculeatus*) whereas surrounding lakes at lower elevations contain, in addition, lake trout (*Salvelinus namaycush*) and brook trout (*S. fontinalis*). Both lakes have a surface area of less than 10 ha and are separated by a distance of about 1 km (Fig. 1). The two lakes belong to different watersheds, Kitturiak being a headwater lake with a limited drainage basin, while Milugiak receives water from a group of small lakes north of Kitturiak. The lakes are covered with ice from November to June and no thermocline forms during summer.

Materials and Methods

Char were collected during four sampling periods: 17 July 1982 – 31 August 1982, 27 June 1983 – 5 July 1983, 27 August 1983 – 12 September 1983, and 26 August 1985 – 6 September 1985. A fish trap was used in Kitturiak in summer 1982 for a mark–recapture experiment to quantitatively assess the char population (Bérubé 1985). Due to the small size of the lake, this assessment was done in order to plan seasonal samples and avoid a premature depletion of its char population. Ninety-three individuals were kept for parasite examination during the mark–recapture study. All other samples from 1982 and 1983 were collected with sinking gill nets (45 × 1.8 m) of 2.5-, 3.8-, 6.3-cm stretched mesh, the nets being installed perpendicular to the shore. Emplacement of the nets in the lakes were the same in the spring and fall of 1983. Very few char were caught at depths greater than 3 m. A total of 392 char in Kitturiak and 388 char in Milugiak were examined for parasites. Fish were processed in a field laboratory immediately after capture to determine sex, fork length, total and eviscerated weight, and gonad weight. Stomach contents were collected and preserved individually in 5% formalin. Viscera were digested with a solution of pepsin and HCl (Meyer and Vik 1963) to recover *Diphyllobothrium* plerocercoids. Identification of plerocercoids was in accordance with descriptions by Andersen (1975) based on general morphology and microtrich length. Representative plerocercoids and adult specimens obtained from experimental infections in hamsters were sent to K. Andersen (Oslo University) for confirmation.

Char otoliths were burned and cracked by applying pressure on the center, as described by Christensen (1964), to examine the annuli on a transverse section. Age interpretations from otolith readings were done according to Nordeng (1961). One otolith per fish was examined subject to two independent readings. Readings disagreed or were indeterminate for 3% of the otoliths.

In 1985, more char were collected to complete the catch curves. Two new mesh sizes were used (3.2 and 5.1 cm) in addition to the previous ones in order to minimize the effect of net selectivity on the catch curves. The two char populations were then fished extensively until almost no fish were caught in the nets. A total of 290 sticklebacks (146 in Kitturiak and 144 in Milugiak) were collected with minnow traps in both lakes from 3 to 10 September 1983. They were preserved in 70% ethanol and later dissected and examined for *Diphyllobothrium*. Zooplankton was collected in July and September 1983 by vertical tow from the lake bottom to surface, using a 30-cm-diameter, 0.23-mm-mesh net.

In the present paper the terms "prevalence," "intensity," and "abundance" are used according to their definitions by Margolis et al. (1982). The terms "spring" and "fall" of 1983 refer to the sampling periods 27 June – 5 July and 27 August – 12 September.

Results

Lake Morphometrics, Physical Chemistry, and Zooplankton

Kitturiak and Milugiak (Fig. 2) have maximum lengths of 705 and 866 m, maximum widths of 218 and 187 m, and perimeters of 1650 and 2520 m, respectively. Shoreline developments (perimeter/$2(\pi \times \text{surface})^{1/2}$) are 1.62 and 2.42. Maximum depth and mean depth are 13 and 3.27 m, respectively, in Kitturiak and 10 and 2.38 m in Milugiak. The surface area and volume are 8.18×10^4 m^2 and 2.69×10^5 m^3 in Kitturiak and 8.69×10^4 m^2 and 2.06×10^5 m^3 in Milugiak. The physical chemistry of both lakes is similar with reference to temperature, conductivity, pH, and dissolved oxygen. Secchi disk transparency is higher in Kitturiak (4.5 m in spring and 5.4 m in fall) than in Milugiak (3.0 and 4.5 m). The plankton faunas are qualitatively similar, containing the cladocerans *Bosminia longirostris*, *Daphnia longiremis* and *Holopedium gibberum* and the copepods *Leptodiaptomus minutus*, *Epischura lacustris*, and *Cyclops scutifer*, an intermediate host of *Diphyllobothrium* (Henricson 1977).

Char Growth and Catch Curves

Length–age relationships for the two populations are shown in the form of growth curves in Fig. 3. Mean lengths for char of all ages are significantly higher in Milugiak than in Kitturiak (t-test, $P < 0.01$). Statistical comparisons were done only for groups containing at least five individuals. The catch curves show a second size mode of 19 cm in Milugiak whereas this second mode is absent in Kitturiak (Fig. 4).

Length–weight relationships are the same in both popu-

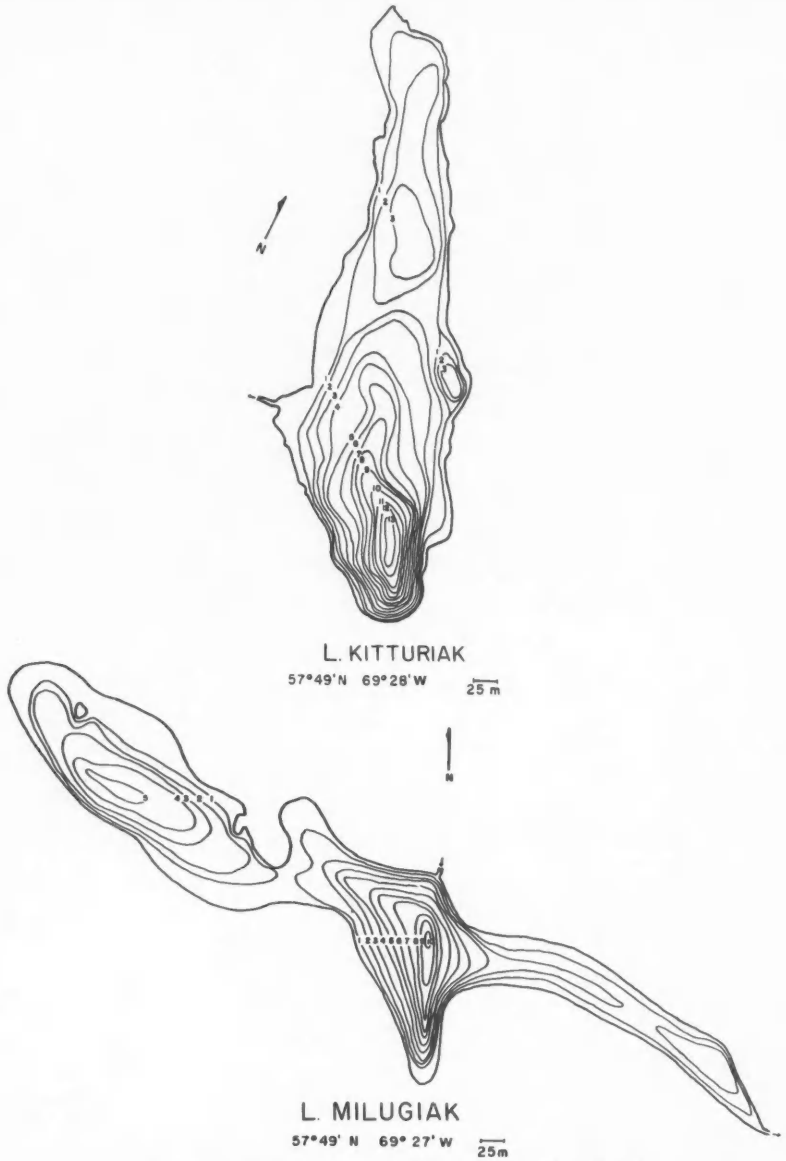

FIG. 2. Bathymetric maps of Lac Kitturiak and Lac Milugiak.

lations (comparison of slopes, $P > 0.05$): Kitturiak: $\log_{10}$ weight $= -5.1438 + 3.0024 \log_{10}$ length ($N = 393$, $r^2 = 0.96$, $P < 0.001$); Milugiak: $\log_{10}$ weight $= -5.1129 + 2.9995 \log_{10}$ length ($N = 392$, $r^2 = 0.98$, $P < 0.001$).

Comparison of Feeding Behavior in the Two Char Populations

Char feeding behavior is described here by reference to the seasonal occurrence of food items in the stomachs. Samples are divided into three size-classes (11–15, 16–20, and >20 cm) and the spring and fall 1983 collections considered separately. The sample size for the largest size-class is suitable for analysis solely in the fall because the 6.3-cm-mesh net was used only then. This largest size-class is almost entirely absent from Kitturiak. Sample sizes and the percentages of fish with empty stomachs are given in Table 1, while the percentages of fish feeding on different food items are presented as histograms in Fig. 5A and 5B. Different foods have been classified according to their origins from limnetic and benthic habitats. Insect pupae are considered here as "limnetic" in accordance with the opinion of Frost (1977) and Hindar and Jonsson (1982) that they are preyed upon mostly during emergence. The coleoptera are terrestrial species and have been classified as "limnetic" because they are presumably caught on the surface of the water. Dytiskid coleoptera were found only in two stomach samples (spring, Kitturiak) and do not appear on the histograms. Statis-

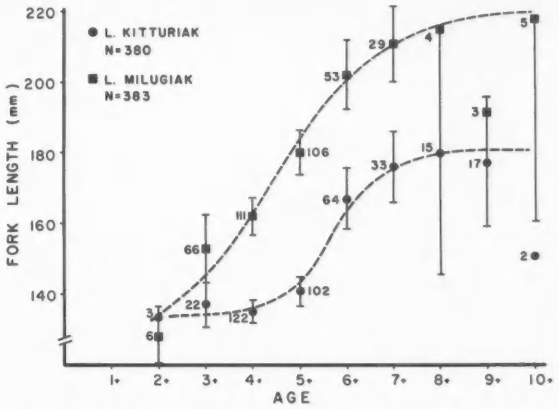

FIG. 3. Growth curves of char. Vertical bars represent 95% confidence intervals (for samples of five individuals and more). The curves are fitted by eye.

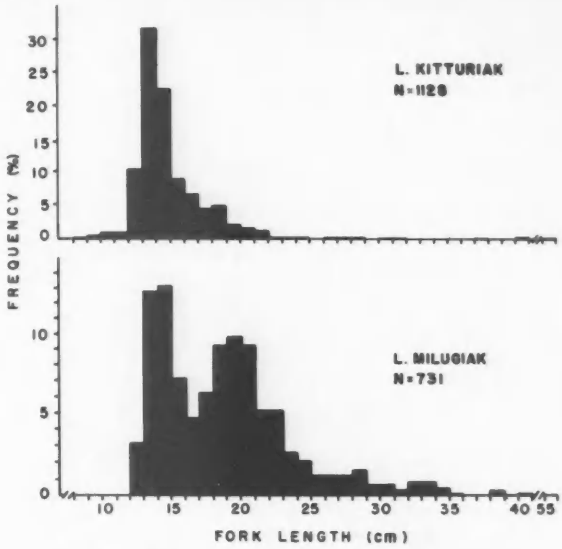

FIG. 4. Catch curves of Lac Kitturiak and Lac Milugiak.

comparisons of food occurrence between the two populations are based on a G test (Sokal and Rohlf 1981).

In the spring (Fig. 5A) the feeding behavior of char in the two lakes is similar. Insect larvae and pupae constitute the two main food items. Some significant differences also appear, however. Cladocerans (mostly *H. gibberum*) are consumed by small char more in Milugiak than in Kitturiak ($P < 0.01$). Coleoptera have also a higher occurrence in Milugiak char of all sizes ($P < 0.01$). Hydracarina are not found in Milugiak samples but are found in more than 25% of the small char in Kitturiak.

In the fall, the situation appears quite different (Fig. 5B). There is a general tendency to concentrate on limnetic and surface foods in Kitturiak and on benthic foods in Milugiak. Consumption of copepods increases significantly ($P < 0.01$) for small char in Kitturiak but remains very low in Milugiak. Sticklebacks are a main food of larger char in Kitturiak but they are significantly less eaten in Milugiak ($P < 0.01$), with occurrences of 46 and 7%, respectively. Only a single large char (40 cm) was found to be cannibalistic, and this individual was from Kitturiak.

Coleoptera and cladocerans disappeared from fish diets in the fall. During this season there is an increase in occurrence of adult insects in stomachs of all char size-classes ($P < 0.01$) in Kitturiak. There is also a decreased consumption of pupae in Milugiak char of all sizes ($P < 0.01$). This results in a higher occurrence of adult insects and pupae in Kitturiak char stomachs in the fall ($P < 0.01$). Conversely, insect larvae are more prevalent in Milugiak char stomachs ($P < 0.05$ for small char and $P < 0.01$ for larger char) as well as amphipods ($P < 0.01$ for small char and $P < 0.05$ for larger char). The overall pattern of difference between the two lakes seems to result from a decreased consumption of insect larvae in Kitturiak and an increase in amphipod consumption in Milugiak. There is a general decrease of feeding activity in Milugiak in fall with 45% of the fish sampled having empty stomachs compared with only 15% in Kitturiak (Table 1). This seems related to an earlier spawning time in Milugiak, as indicated by the presence of eggs occurring in stomachs there.

Infection Levels

Prevalences of *D. ditremum* are 78 and 18% in Kitturiak and Milugiak, respectively (Table 2). The frequency distributions are highly overdispersed (Fig. 6). When subdivided into the three size-classes, the distribution remains overdispersed. For this reason the Mann–Whitney test has been used for all comparisons of plerocercoid abundance. Abundance of *D. ditremum* is significantly higher ($P < 0.01$) in Kitturiak than in Milugiak for the populations considered as a whole or by size-classes (Table 2). The frequency distribution in Kitturiak (Fig. 6) shows that four individuals are much more heavily infected than the others, with intensities of 800 plerocercoids or more. However, when these four individuals are excluded from the sample, the abundance remains significantly higher ($P < 0.01$) in Kitturiak (27.7 plerocercoids/fish) than in Milugiak (1.2 plerocercoids/fish). No significant difference in plerocercoid abundance is found between males and females in either lake. Sticklebacks also appear more heavily infected in Kitturiak than in Milugiak. Prevalences are 48 and 29%, and abundance is significantly higher ($P < 0.01$) in Kitturiak (1.4 vs. 0.5 plerocercoids/fish).

Abundance of *D. ditremum* in char increases with age as well as with length in both lakes. As few fish are infected in Milugiak, it is difficult to establish an acquisition model for tha. population. In Kitturiak, however, the increase of abundance with age roughly follows a quadratic curve (Fig. 7) with no indication of levelling off at maximum abundance. Variance to mean ratios increase with age but prevalences remain at about 80% throughout. There was a similar pattern of plerocercoid acquisition with the length of host fish. The curve of plerocercoid abundance versus age suggests that there is no increase between age 3+ and age 5+. To evaluate acquisition within one season, plerocercoid abundance of three char age-classes (3+ to 5+, 6+ to 8+, and >8+) was compared between the spring and fall of 1983 (Table 3). Since gill nets of 6.3-cm mesh size were used only during the fall, fish caught with such gear were omitted from the comparison. Abundance does not significantly change between spring and fall for the 3+ to 5+ age-class but increases significantly ($P < 0.01$) for the 6+ to 8+ age-class, from 25.6 to 62.1 plerocercoids/fish.

TABLE 1. Number of char stomachs examined and number of empty stomachs per sample in 1983 in Kitturiak and Milugiak.

Lake	Char length group (cm)	Spring			Fall		
		N	Number of empty stomachs	%	*N*	Number of empty stomachs	%
Kitturiak	11–15	89	11	12.4	122	6	4.9
	16–20	47	9	19.1	33	5	15.2
	>20	2	1	50.0	6	0	0
	Total	138	21	15.2	161	11	6.8
Milugiak	11–15	43	7	16.3	114	64	56.1
	16–20	51	11	21.6	82	36	43.9
	>20	10	3	30.0	43	9	20.9
	Total	104	21	20.2	239	109	45.6

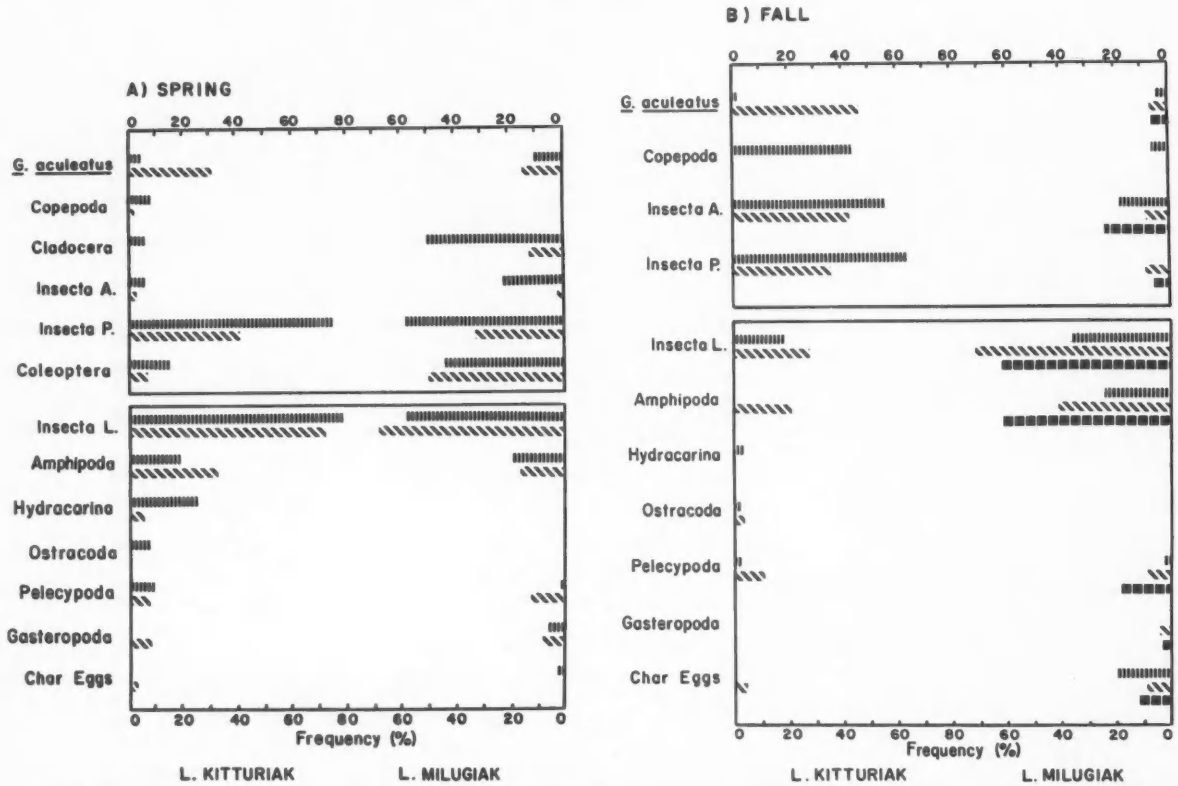

FIG. 5. Feeding behavior of char in (A) spring 1983 and (B) fall 1983. Results are expressed as percentages of fish feeding on different food items. The upper panel contains items found in the limnetic zone or at the water surface. The lower panel contains items found in the benthos. Char are divided into size-classes 11–15 cm (▮▮▮▮▮▮), 16–20 cm (▮▮▮), and >20 cm (▮▮▮).

Abundance in the >8+ age-class does not change significantly. The apparent decrease observed is due to one highly infected char caught in the spring.

Diphyllobothrium ditremum Abundance as Related to Copepod and Stickleback Consumption

Copepods are a known source of *D. ditremum* infection for fish. Sticklebacks as a source of infection necessitate the capacity of the plerocercoid to reestablish in the viscera of the predatory fish. This capacity of transmitting from fish to fish has been verified experimentally for *D. dendriticum* (Unsworth 1944; Vik 1957; Bylund 1972; Halvorsen 1970; Halvorsen and Wissler 1973). Halvorsen and Wissler (1973), however, questioned the capacity of *D. ditremum* to reestablish in a predatory fish host.

Here, we verify that the presence of sticklebacks or copepods in the stomachs of char is associated with a higher abundance of *D. ditremum*, and establish that these food items are a source of infection in Kitturiak. Relevant information is provided in

TABLE 2. Prevalence, abundance, and variance to mean ratio of *D. ditremum* in Kitturiak and Milugiak.

Char length group (cm)	Kitturiak				Milugiak			
	N	Prevalence (%)	Abundance ($\bar{X}$)	$S^2/\bar{X}$	*N*	Prevalence (%)	Abundance ($\bar{X}$)	$S^2/\bar{X}$
11–15	262	79.0	12.9	20.4	154	14.3	0.4	8.4
16–20	116	74.1	50.8	89.4	144	19.5	0.5	3.6
>20	10[a]	90.0	149.6	95.8	90	22.2	3.7	56.5
Total	388	77.8	27.7	92.0	388	18.0	1.2	44.7

[a]Does not include the four individuals with 800 and more plerocercoids in Kitturiak.

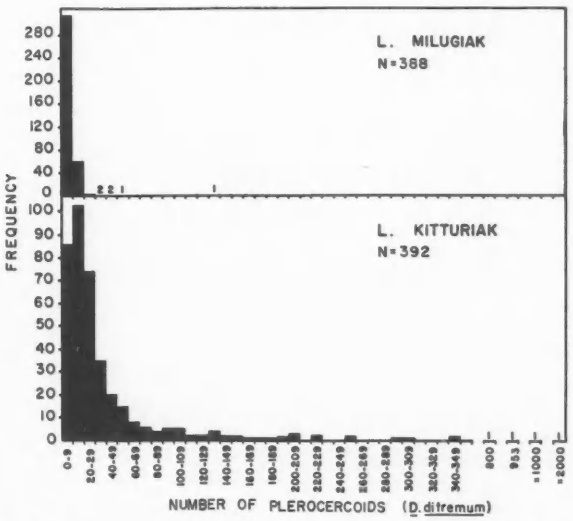

FIG. 6. Frequency distributions of *D. ditremum* in char in Lac Kitturiak and Lac Milugiak.

Table 4. The groups of individuals found with copepods or sticklebacks in their stomachs have a higher abundance of plerocercoids than the fish found feeding on other food items ($P < 0.01$ in both cases). The increase of abundance with consumption of sticklebacks is particularly high (from 11.7 to 95.4 plerocercoids/fish) even if two very highly infected piscivorous fish (with intensities of about 1000 and 2000 worms) are excluded from the analysis.

Discussion

On the basis of the above results, it appears that the observed differences in abundance of *D. ditremum* in Kitturiak and Milugiak are related to differing feeding patterns among their char populations, these patterns in turn being determined by fundamental properties of food webs in the two lakes. Although arctic char are opportunistic feeders, they show preference for certain food items such as large planktonic crustaceans, amphipods, and other large invertebrates (Johnson 1980) whereas other food items such as copepods are often not much utilized (Frost 1977). From our information on stomach content analysis, Milugiak seems to provide better feeding conditions for char than does Kitturiak. This view is reinforced by the higher growth rate of char in Milugiak. Preferred food items such as cladocerans and amphipods are extensively consumed in

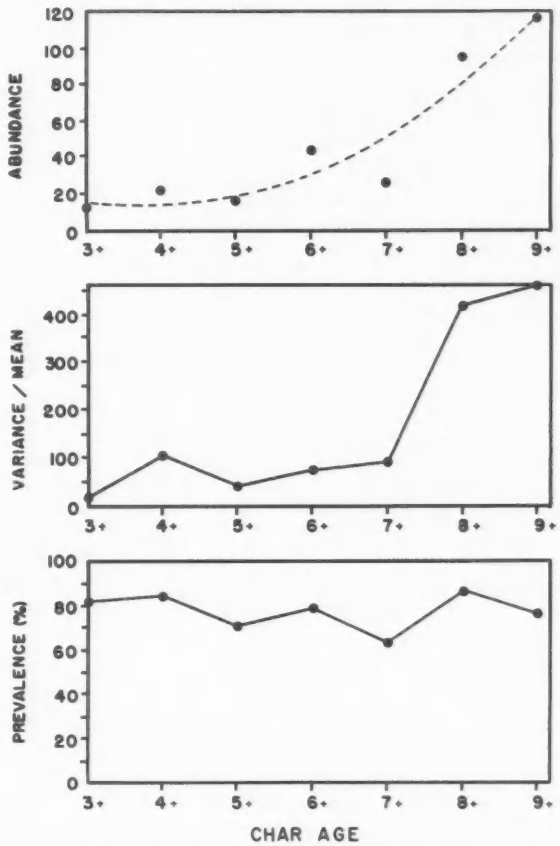

FIG. 7. Abundance, variance to mean ratio, and prevalence of *D. ditremum* with age of char. The curve of abundance vs. age follows a quadratic formula: $Y = 356 - 134X + 12.8X^2$ ($N = 375$, $r^2 = 0.42$, $P < 0.001$).

Milugiak whereas less desirable prey organisms such as copepods are more important to the char of Kitturiak.

Increased consumption of amphipods in the autumn (as in Milugiak) has previously been reported in the Ungava at Lac Noname (Fraser and Power 1984), but this does not occur in Kitturiak. Instead, there is an increase in planktonic feeding and diminution of benthic feeding. Such a phenomenon was reported in a landlocked char population of Newfoundland (Rombough et al. 1978) where the char are also characterized by a slow growth rate as a result of poor feeding conditions.

TABLE 3. Prevalence, abundance, and variance to mean ratio of *D. ditremum* in Lac Kitturiak in the spring and fall of 1983.

Char age group	Spring (27 June – 5 July)				Fall (27 August – 12 September)			
	N	Prevalence (%)	Abundance ($\bar{X}$)	$S^2/\bar{X}$	*N*	Prevalence (%)	Abundance ($\bar{X}$)	$S^2/\bar{X}$
3+ to 5+	73	74.0	15.0	52.0	122	79.6	12.4	14.0
6+ to 8+	53	64.2	25.6	67.0	26	80.8	62.1	91.4
>8+	7	57.1	174.4	722.4	6	33.3	78.9	87.6

TABLE 4. Abundance and variance to mean ratio of *D. ditremum* in char feeding on copepods or sticklebacks in Kitturiak and Milugiak.

Char length group (cm)	Prey	Kitturiak			Milugiak		
		N	$\bar{X}$	$S^2/\bar{X}$	*N*	$\bar{X}$	$S^2/\bar{X}$
11–15[a]	Copepods (–)	65	7.1	12.0	43	0.1	1.2
11–15[a]	Copepods (+)	49	18.6	5.6	3	6.6	20.0
>15[b]	Sticklebacks (–)	43	11.7	37.6	112	0.3	5.3
>15[b]	Sticklebacks (+)	29	95.4	67.6	13	2.0	2.5

[a] In order to minimize the effect of fish size in the comparison of plerocercoid abundance between copepod and noncopepod feeders (not comprising fish with empty stomachs), only the small size-class of char was considered. Also, only the fall sample was used because copepods seem to be available mainly in this season.

[b] Only the bigger size-class is considered. The piscivorous individuals with 800 plerocercoids and more are excluded from the comparison.

Differences in food availability between nearby lakes have been reported in the Northwest Territories (Johnson 1980) and in Newfoundland (Leggett and Power 1969). Because most of the productivity of northern lakes occurs in the benthos, Johnson (1980) believes that the nature of the shoreline contour is of prime importance in determing productivity and abundance of fish food. It is probable that Kitturiak, because of its poor shoreline development and relatively greater mean depth, does not favor production of benthic organisms to the same extent as Milugiak. As a headwater lake, Kitturiak also receives very little allochtonous matter. In Milugiak, evidence for receiving material from its larger watershed is shown by the abundance of terrestrial coleoptera (*Amara alpina*, *Pterostichus haematopus*, and *P. brevicornis*) occurring as fish food in the spring. These are flightless carabids (Lindroth 1966, 1968) which are transported into lakes as a result of spring flooding.

Other explanations could account for differences in parasite abundance, feeding behavior, and growth rates in two char populations. Henricson and Nyman (1976) have related similar findings to distinct char genotypes, although their study applied to sympatric populations. Allopatric populations with different genotypes are not likely to be found in neighboring lakes such as those studied here. Moreover, Nordeng (1983) has demonstrated that phenotypic expression of different char genotypes is itself dependent on food availability. Another explanation for the differences in *Diphyllobothrium* abundance would be that the two lakes are differentially used by piscivorous birds. However, differential use of lakes by birds is unnecessary to account for the observed variations in parasite abundance, when considering the very significant differences in char feeding behavior. Even if greater bird presence were to result in more infected copepods and sticklebacks, this would be of limited importance for the char of Milugiak, which do not much exploit such food organisms.

According to a model by Kerr (1971), the adoption of piscivorous habits can occur when the cost of searching for small prey becomes higher than the energy gained by their consumption. In Kitturiak, it appears that the selective pressure to become piscivorous, due to a low density of invertebrates, is higher than in Milugiak, where few fish eat sticklebacks and where growth is rapid. Although stickleback populations have not been quantitatively assessed, they appeared very abundant along the shore of both lakes, and were readily available prey for char due to their high *Schistocephalus solidus* infections (77% in Kitturiak and 67% in Milugiak). This very large cestode has debilitating effects on sticklebacks and has been found experimentally to increase host susceptibility to predation (Giles 1983). However, stickleback consumption does not seem to provide improved growth to Kitturiak char, which fail to accumulate around a second length mode at 19 cm as do those in Milugiak. When comparing two populations of landlocked Atlantic salmon (*Salmo salar*) with different growth rates, Leggett and Power (1969) reported faster growth associated with stickleback feeding. In the present case the char may not survive very long on a stickleback diet, once becoming heavily infected with *Diphyllobothrium*.

Indirect evidence of *Diphyllobothrium*-induced char mortality has been found for char populations of Sweden (Henricson 1977, 1978) and Baffin Island (Curtis 1984). Both authors observed a plateau of abundance with age at a level of around 100 plerocercoids/fish (after ages 8+ and 11+, respectively) and patterns of seasonal variation that could be explained by the death of heavily infected hosts. The maximum abundance at-

tained with age in Kitturiak is also around 100 plerocercoids/fish. However, no plateau of abundance was noticed here because older age-classes, such as those found by Henricson (1977, 1978) and Curtis (1984), were missing. Halvorsen and Andersen (1984) recorded a decrease in abundance at ages 9+ and >9+, although their sample sizes for those age-classes were very small.

The *D. ditremum* acquisition curve for Kitturiak char registers a sharp increase at the age when most fish first are able to become piscivorous. Starting at age 6+, parasite abundance is seen to rapidly increase. Halvorsen and Wissler (1973) have conducted experiments in which *D. ditremum* plerocercoids from char failed to reestablish in rainbow trout (*Salmo gairdneri*). In our study, consumption of sticklebacks is associated with higher *Diphyllobothrium* abundance in char. This suggests that the success of fish to fish transmission is probably dependent on fish species. The role of sticklebacks in the transmission of *D. ditremum* can be increasingly important when benthic food is rare, as in Kitturiak. Sticklebacks are opportunistic feeders which are often found in competition with young salmonids (Wootton 1976). This possibly accounts for sticklebacks in Kitturiak being more heavily and frequently infected than the ones in Milugiak.

That the presence of sticklebacks or copepods in the Kitturiak char stomachs is significantly associated with high levels of infection implies some degree of constancy in the diet of individual char. This would account, in part, for the observed overdispersion of plerocercoids in the char population. Our results agree with those of Halvorsen and Andersen (1984), who related overdispersion of *D. ditremum* in char to an overdispersion of copepods in char stomachs, which implies that some individuals feed more extensively on this food item than others.

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Woody Debris as a Source of Fine Particulate Organic Matter in Coniferous Forest Stream Ecosystems[1]

G. Milton Ward

Aquatic Biology Program, Department of Biology, University of Alabama, University, AL 35486, USA

and Nicholas G. Aumen

Department of Biology, University of Mississippi, University, MS 38677, USA

Ward, G. M., and N. G. Aumen. 1986. Woody debris as a source of fine particulate organic matter in coniferous forest stream ecosystems. Can. J. Fish. Aquat. Sci. 43: 1635–1642.

The potential contribution of woody debris to fine particulate organic matter pools (0.45 μm ≤ FPOM < 1 mm) was investigated in a coniferous forest stream ecosystem in western Oregon. The amount of wetted surface area of both large (>10 cm) and fine woody debris (1–10 cm) was 0.018 and 0.069 $m^2 \cdot m^{-2}$ stream bed, respectively, during summer base flow. These values increase to 0.062 and 0.195 $m^2 \cdot m^{-2}$ stream bed during winter flows. Studies of vertical distribution indicated that most fine wood is concentrated within 0.3 m of the stream bottom, while large wood is more evenly distributed up to 0.7 m. Lignin concentrations of large wood, soil, and FPOM were very similar. Examination of FPOM samples with scanning electron microscopy revealed an abundance of wood-derived particles. Erosion rates of wood surfaces ranged between 0.5 and 11 $mm \cdot yr^{-1}$ depending on decay state of the log. Using conservative estimates of processing rates, woody debris could be a source for approximately 90 $g \cdot m^{-2} \cdot yr^{-1}$ of FPOM, but with slightly a less conservative estimate, wood processing could easily generate several times the FPOM that is contributed by leaf and needle litter.

L'apport des débris ligneux à la matière organique particulaire (0,45 μm ≤ MOP < 1 mm) a fait l'objet d'une étude portant sur un écosystème de cours d'eau de forêt de conifères de l'ouest de l'Orégon. La surface mouillée des débris ligneux grossiers (> 10 cm) et fins (1–10 cm) était, respectivement, de 0,018 et 0,069 m^2 par mètre carré de lit de cours d'eau au cours de l'écoulement de base d'été. Ces valeurs atteignaient 0,062 et 0,195 m^2 par mètre carré au cours des écoulements d'hiver. L'étude de la distribution verticale a montré que la plus grande partie des débris fins était concentrée à moins de 0,3 m du fond du cours d'eau tandis que les débris plus gros étaient distribués plus également à moins de 0,7 m. Les teneurs en lignine des gros débris de bois, du sol et de la MOP étaient très semblables. L'examen d'échantillons de MOP au microscope électronique à balayage a montré une grande abondance de particules ligneuses. Le taux d'érosion des surfaces ligneuses variait de 0,5 à 11 mm par an tout dépendant de l'état de décomposition de la bille. Des estimations prudentes des taux de transformation indiquent que les débris de bois pourraient être une source d'environ 90 $g \cdot m^{-2}$ par an de MOP. L'utilisation d'estimations légèrement moins conservatrices indique cependant que la transformation du bois pourrait facilement être la source d'une quantité de MOP équivalent à plusieurs fois celle produite par la litière de feuilles et d'aiguilles.

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Over the past several years the importance of woody debris in the ecology of small, high-gradient streams (Bilby and Likens 1980; Triska and Cromack 1980) and large, lowland rivers (Sedell and Froggatt 1984; Triska 1984; Wallace and Benke 1984) has become well recognized. Large woody debris (LWD, >10 cm) is important in shaping channel structure (Swanson and Lienkaemper 1978), in retention of inorganic sediment, leaves, and fine particulate organic matter (Bilby 1981; Speaker et al. 1984), and in the formation of habitat and food for fish and invertebrates (Hall and Baker 1977; Sedell and Triska 1977; Benke et al. 1985).

Wood debris is also an important component of organic matter budgets in stream ecosystems, particularly those in the Cascade Mountains of Oregon where accumulations are quite large. On a mass basis, loadings of all LWD lying within the channel (both submerged and emergent) range from 43 $kg \cdot m^{-2}$ in first-order channels to 28 $kg \cdot m^{-2}$ in third-order streams (Keller and Swanson 1979). Fine woody debris (FWD, 1–10 cm) is less abundant (0.61 $kg \cdot m^{-2}$; Triska and Cromack 1980) than LWD, but is a primary food source for xylophagous insects (Anderson et al. 1978, 1984) and has the potential to contribute substantially to energy flow and nutrient cycling as a result of its large nutrient pool and its intermediate availability between leaves and large wood (Triska and Cromack 1980).

Like leaf and needle litter, woody debris also has the potential to be a source of fine particulate organic matter (0.45 μm

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≤ FPOM < 1 mm). Cummins et al. (1983) speculated that FPOM generation from wood could be an important detrital process even in systems where wood is not overly abundant. However, the generalized view of coniferous forest stream structure and function given by Triska et al. (1982) did not include FPOM resulting from LWD processing. Production of FPOM from wood most likely results from a combination of microbial activity and physical abrasion from the current. Preliminary observations in Mack Creek revealed the presence of many logs that possessed relatively soft surface wood. The exposure of these wood surfaces to highly turbulent water during winter base flow periods, and particularly during storm flows, could produce FPOM through fragmentation.

The potential significance of large and fine woody debris as a source of FPOM is likely to be proportional to the quantity of submerged wood in a stream channel as well as the rate at which wood surfaces are fragmented. Previous estimates of woody debris standing crop in Cascade Mountain streams (e.g. Anderson et al. 1978; Keller and Swanson 1979) have included all wood within the channel, whether potentially in contact with the current or not. Thus, more precise measurements of submerged wood surface area are needed for calculations of FPOM production. The quantities of FPOM generated from wood will also be a function of the rate at which microbial decomposition softens the wood surfaces. Estimates of LWD processing rates are at best only speculative, and turnover rates are certainly measured in decades, if not centuries. Processing rates of FWD are known to be greater than those for LWD (Triska and Cromack 1980), and processing rates of FWD are faster for wood with high surface to volume ratio, such as chips and twigs (Triska and Cromack 1980). However, at present there are no estimates of FPOM production for either FWD or LWD. In this study we have sought to provide evidence that woody debris can be a significant source of FPOM to the Mack Creek system. To do this, we determined the quantities of woody debris submerged during summer and winter, estimated losses of wood from LWD surfaces, compared the lignin and cellulose content of woody debris, soil, and FPOM, and examined FPOM samples for the presence of wood-derived particles.

Study Site

All measurements and sample collections were conducted in Mack Creek, a third-order channel located at 830 m elevation in the H. J. Andrews Experimental Ecological Reserve in the Western Cascade Mountains of Oregon. Mack Creek is a high-gradient (10%), bedrock-constrained system that drains a 6.0-km^2 watershed. The channel morphology consists of stair-steps of pools, free-fall zones, and turbulent water around boulders (Triska et al. 1982). The surrounding forest is composed of massive, old-growth Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) that typically reach heights of 60 m. Leaf and needle litter inputs from riparian areas are approximately 360 g·m^{-2}·yr^{-1} (S. V. Gregory, Oregon State University, Corvallis, OR, unpubl. data).

The most apparent physical feature of the Mack Creek stream channel is the large accumulations of woody debris. Because of the large diameter (often >0.5 m) and long length of Douglas-fir trees, a narrow channel width, and the steep side slopes, logs often fall with one end in contact with the streambed and the other extending many metres above the channel bottom. Additionally, storm flows often dislodge even large debris, repositioning the pieces in large accumulations or depositing them outside the active channel. As a result, relatively few logs are in contact with the stream bottom along their entire length, and much of the large woody debris is located above summer base flow.

At summer low flow, the average water depth in Mack Creek is <0.1 m, with many areas of stream bottom barely wetted. However, winter base flow is approximately 0.4 m higher than summer (George W. Lienkaemper, Forestry Sciences Laboratory, Corvallis, OR, pers. comm.) as a result of the seasonal concentration of precipitation during November–April. Because of the very shallow water depths and large size of the debris, few logs are completely submerged in summer, and large debris projects from the stream bottom to a point well above winter stage heights.

Materials and Methods

Surface area and mass of LWD was determined for all 100 logs present in a 220-m reach of Mack Creek where the average channel width was 10 m. Measurements of log diameter, length, and height above the stream bottom were conducted in July of 1985 at summer low flow in order to determine the minimum surface area that was wetted during the annual hydrologic cycle. Only logs located in the active channel were selected and measurements were restricted to the portion of each log situated below a 1.5-m height above summer base flow. The 1.5-m height was selected for being well above Mack Creek's winter discharge. The remaining portion of each log above the 1.5-m mark often continued many metres up the hillslope and would rarely be affected by stream flow. Surface areas were calculated for wetted portions of each log at summer base flow and at the projected winter flow. Surface area data were divided into 10-cm vertical increments to illustrate vertical distribution patterns of large woody debris. Mass was calculated from volume data using a density of 0.361 g·cm^{-3} (SD = 0.042, n = 8). Densities were determined empirically (Hg displacement) for randomly collected pieces of FWD and for samples from surfaces of LWD.

Surface area of FWD was determined by sampling ten 2-m-wide transects evenly spaced along a 100-m reach of Mack Creek. Within each transect, diameters and lengths of each piece of fine wood (approximately 1000) were measured and the vertical distribution was noted up to 0.7 m above summer base flow. Preliminary observations indicated that most of the fine wood located in the active channel was situated in this vertical region. Each piece was assumed to be either a rectangular solid or a right circular cylinder and the appropriate calculations for surface area and volume applied. Mass was calculated from volume estimates using a density of 0.395 g·cm^{-3} (SD = 0.126, n = 16).

One of the most likely sources of wood-derived FPOM is from the surfaces of microbially processed logs that are subjected to physical abrasion from stream flow. In order to document LWD processing, reductions in the cross-sectional area of several Douglas-fir logs influenced by stream current were observed over a period of several years. Five logs ranging in length from 3.7 to 5.1 m and in diameter from 24 to 70 cm were initially chosen for study. Each had fallen into the channel some years prior to the beginning of the study and already had the bark removed by natural processes. No accurate estimates could be made of the previous residence time of these logs in the channel, but they represented a wide range of decay, from soft, punky wood to very sound wood. For the purposes of this

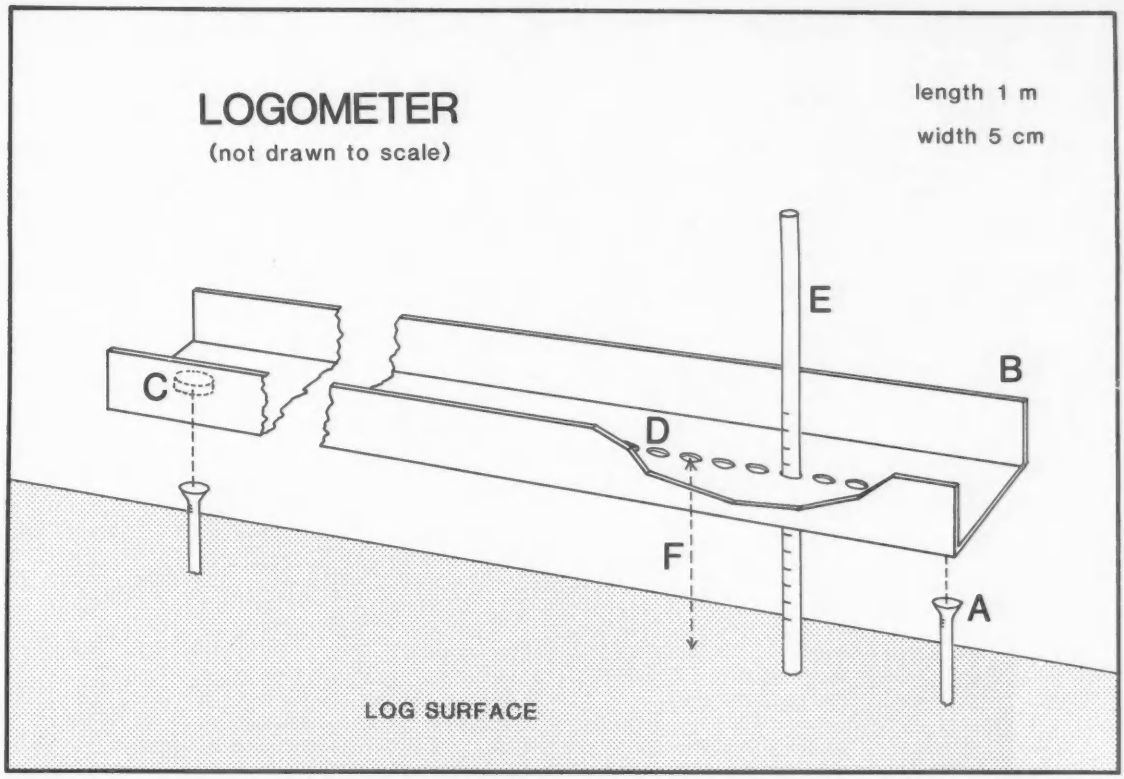

FIG. 1. Cut-away drawing of "logometer" used to determine surface wood loss from decomposing logs. Galvanized spikes (A) (18 cm) are driven into the study log at approximately 1-m intervals with the top 3–5 cm of each spike left exposed. The spikes serve as permanent reference points for positioning the logometer (B), with the spike heads fitting into receptacles (C) at either end of the instrument. This allows the logometer to be repositioned in exactly the same place each time the log is measured. The body of the logometer is constructed of 3-mm aluminum plate with holes (D) drilled through the bottom at 2-cm intervals. These holes serve as guides for a rod graduated in 1-mm increments (E) used to determine the distance (F) between the log surface and the bottom of the instrument. This distance is determined at each of the holes along a 2-m transect of the study log, and can be remeasured repeatedly and accurately over a long-term study period (years).

study, each log was reoriented perpendicular to the current to simulate naturally occurring logs that receive substantial stream flow. Over the winters of 1983 and 1984, four of the five logs were repositioned out of the active channel by storm flows and only one log remained in its original position throughout the entire experiment. Logs were first measured in August of 1982 and remeasured in October of that year. Logs that remained in the active channel were again measured in the fall of 1983 and 1984 and July of 1985. The study logs were monitored yearly by measuring changes in their surface profile using the "logometer" illustrated in Fig. 1. Galvanized spikes (18 cm) were driven into the logs as reference points and holdfasts for the instrument. Detailed measurements of changes in the surface profile of each log could be made by situating the logometer on the spikes. Any loss from the surface profile due to processing would be reflected in an increased distance from the instrument to the surface of the log. A 2-m transect of each log was measured for changes at 2-cm intervals, and it was determined that these measurements were reproducible to be <2 mm. The mean change in distance from the logometer to the surface of the log was calculated based on 92 points along the transect.

Surface scrapings were collected from the five study logs when they were originally placed in their new positions in August of 1982. Lignin and cellulose concentrations of the surface wood were determined as described below, and samples were obtained for [^{14}C]lignocellulose decomposition experiments as described in Aumen et al. (1983) according to the methods of Crawford (1981). Briefly, scrapings were homogenized and placed in incubation bottles with ^{14}C-labeled natural lignin and cellulose prepared from Douglas-fir. The microbial decomposition rates of lignin and cellulose on the wood scrapings were determined by monitoring the evolution of $^{14}CO_2$ over a 3-wk incubation period in the laboratory.

Collections of FPOM for lignin and cellulose determinations were obtained from a series of backwater and pool habitats in Mack Creek by wet-sieving sediment detritus through a series of standard sieves. Soil samples were likewise collected from the riparian zone immediately adjacent to the active channel. Surface wood scrapings approximately 1 mm thick were obtained from a series of downed Douglas-fir logs in Mack Creek using a standard carpentry plane as described by Aumen et al. (1983). Fine wood samples were collected from the belt transect studies described above. All samples were dried at 50°C and the wood scrapings and fine wood were ground to pass a 0.85-mm sieve (No. 20 mesh). Wood scrapings, fine wood,

TABLE 1. Seasonal comparison of the surface area and mass of submerged large (>10 cm in diameter) and fine wood (1−10 cm) in a 220-m reach of third-order Mack Creek. Winter flow is defined as a stage height 0.4 m above low summer flow. Estimates of large wood mass and surface area are from a single 220-m reach and thus, no error terms are available.

	Summer base flow		Winter base flow	
	Average	SD	Average	SD
Large wood surface ($m^2 \cdot m^{-2}$ stream bed)	0.018	—	0.062	—
Fine wood surface ($m^2 \cdot m^{-2}$ stream bed)	0.069	0.113	0.195	0.138
Total wood surface ($m^2 \cdot m^{-2}$ stream bed)	0.087	—	0.257	—
Large wood mass ($kg \cdot m^{-2}$)	0.871	—	10.975	—
Fine wood mass ($kg \cdot m^{-2}$)	0.212	0.284	1.03	0.868
Total wood mass ($kg \cdot m^{-2}$)	1.083	—	12.005	—

soil, and FPOM samples were analyzed for cellulose and acid-detergent lignin content after the method of Goering and van Soest (1970) as modified by Mould and Robbins (1981). Results were expressed as a percent of ash-free dry weight (AFDW) after correcting for insoluble ash and losses of acid-soluble ash.

Soil and FPOM samples for scanning electron microscopy (SEM) were preserved in 1.5% glutaraldehyde, fixed in 1% OsO_4, and dehydrated in a graded ethanol series. Samples were dried using a Denton Vacuum DCP-1 critical point dryer, coated with 10-nm-thick Au/Pd wire using a Technics Hummer V, and examined with a ETEC Autoscan SEM. The percentage of wood particles in FPOM samples was determined by examining at least 100 FPOM particles in each of three size classes (1.0−0.5 mm, 0.25−0.1, and 0.1−0.053 mm) with SEM at magnifications >300× and determining the percentage of recognizable wood fragments in the total number of particles examined.

Results

Vertical Distribution of Wood

The vertical distribution of LWD surface area is rather even (Fig. 2). There are, however, somewhat greater amounts at summer and winter base flow levels. During summer low flow, approximately 0.018 m^2 of LWD surface is submerged per square metre of stream bottom, but during winter, submerged surface area increases by 3.5 times to 0.062 $m^2 \cdot m^{-2}$ stream bottom (Table 1). Storm flows would, of course, result in even higher water levels and more submerged wood. With an increase of 20 cm above normal winter base flow, there would be an additional 40% increase in submerged surface area.

In contrast with LWD, FWD surface area is concentrated within 0.3 m of the stream bottom (Fig. 2). Approximately 0.069 m^2 of FWD is submerged per square metre of stream bottom during summer low flow, but at winter, base flow surface area increases by a factor of 2.8 to 0.195 $m^2 \cdot m^{-2}$ stream bottom (Table 1). The quantity submerged in summer represents 36% of the total fine wood surface area submerged at winter base flow, but with only a 10-cm rise in stage, more

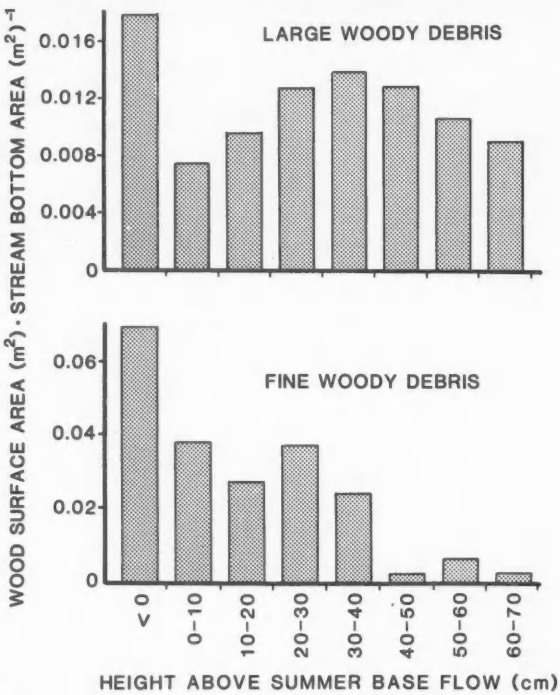

FIG. 2. Vertical distribution of large (>10 cm in diameter) and fine (1−10 cm) wood in Mack Creek. Arrows indicate typical summer and winter base flow levels.

than 50% is submerged. Above 0.4 m, quantities of fine wood surface area decline sharply (Fig. 2). Storm flows up to 0.7 m would therefore submerge little more fine wood than is wetted at winter base flow.

There is much more wetted surface area in FWD as compared with LWD (Table 1). During the summer, FWD surface area is 12 times larger than that of large wood (79% of the total submerged wood surface is FWD), although the relative difference declines to 3 times during winter. Further increases in stage height, such as occurs during storm flows, would make the difference even smaller.

Large and fine wood mass is more evenly distributed vertically than is surface area. Approximately 0.2 $kg \cdot m^{-2}$ is submerged during summer, but that increases by 5 times during winter (Table 1). Much of the fine wood submerged during summer is relatively small pieces with high surface to mass ratios, but wood located above the streambed near winter base flow heights tends to be larger. LWD mass submerged at winter flow is approximately 10 times that wetted at summer flow (Table 1).

Production of FPOM from Wood Surfaces

At the beginning of the experiment, log A was considerably more processed than log B and possessed a surface with much softer wood. Lignin values for the two logs were somewhat similar, with log A containing 30.6% lignin and log B containing 27.3% lignin; however, the surface wood of log A contained 33.9% cellulose, while log B contained 41.5% cellulose, similar to that of living Douglas-fir (Table 2). The dissimilarity in substrate composition may be explained by differ-

TABLE 2. Lignin and cellulose content (% of AFDW) of FPOM and potential sources of FPOM in an old-growth, conifer-dominated reach of Mack Creek.

Organic material	% lignin	SD	% cellulose	SD
Sound Douglas-fir bole wood	26.8	1.95	42.4	1.63
Fresh Douglas-fir chips[a]	30.0	—	48	—
Fresh Douglas-fir twigs[a]	33.0	—	40	—
Large woody debris (>10 cm)[b]	43.4	16.28	27.4	13.2
Douglas-fir needles[c]	24.5	—	14.5	—
Red alder (*Alnus rubra*)[c]	9.5	—	9.0	—
Vine maple (*Acer circinatum*)[c]	8.5	—	14.7	—
Fine woody debris	59.1	6.83	18.1	2.29
FPOM (0.01−1 mm)	44.7	5.16	13.2	2.92
Soil (0.1−1 mm)	43.4	8.19	10.9	3.74

[a]F. J. Triska, unpublished data.
[b]Average of 27 logs from a variety of age and decay classes.
[c]Triska et al. (1975).

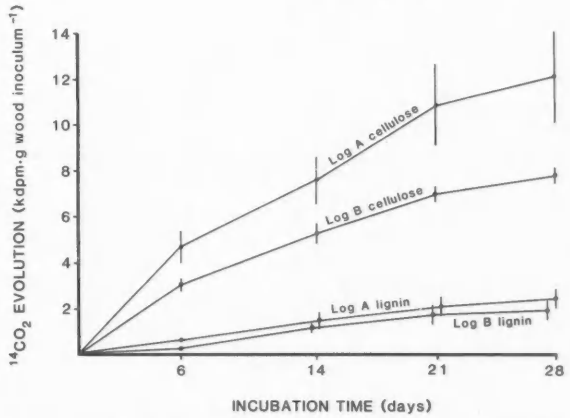

FIG. 3. Decomposition of [^{14}C]lignocelluloses in surface wood samples obtained from logs A and B (see text) in October of 1982. Bars indicate standard errors of the means.

ences in lignocellulose processing rates. Figure 3 illustrates that the two logs had similar rates of lignin decomposition, but that log A had a considerably faster rate of cellulose mineralization.

Logometer data demonstrated that measurable amounts of wood were lost from the surface profiles of logs A and B during the study period (Fig. 4). Log A, with its softer and more decomposed surface, exhibited the greater losses. Portions of the surface of log A lost up to 6.3 cm of wood in 1 yr. The average loss was 11.1 mm·yr^{-1}. While it is unknown if this wood was removed all as large pieces or as FPOM, it is clear that the wood surface experienced significant erosion.

In contrast, log B had relatively sound surface wood and exhibited a much lower processing rate (Fig. 4). Slight losses of wood occurred all along the surface profile, and after 3 yr the average loss at each point along the profile was 0.5 mm·yr^{-1}. The rate loss for this log was near the lower limits of detection with this technique; however, comparison with a control is possible. A third log was initially measured along with the others, but unlike the rest, never came into contact with the stream. The initial profile and one measured a year later were almost identical. It thus appears that processing does occur even in rather sound wood.

Average depth of wood loss for the two logs ranged from <1 to 11 mm·yr^{-1}. These values represent logs within different decay states. Based on this range, we have calculated the potential quantities of FPOM produced through woody debris processing. However, our calculations must be considered preliminary given the limited number of logs measured and the limited knowledge of FWD processing rates. Nevertheless, these calculations suggest that substantial inputs of woody FPOM are possible even with conservative estimates of wood surface erosion. For example, given 0.062 m^2 LWD surface·m^{-2} stream bottom (Table 2), and assuming that all woody debris is relatively sound, losing only 1 mm·yr^{-1} (all as FPOM), approximately 22 g·m^{-2}·yr^{-1} would be transferred to the FPOM pool. Assuming this same loss rate for fine wood, inputs would be approximately 77 g·m^{-2}·yr^{-1}. Together, losses from woody debris surfaces may at a minimum be 2 times the mean FPOM standing crop in Mack Creek (Naiman and Sedell 1979), and equivalent to approximately 50% of FPOM generated annually from leaf and needle litter inputs (assuming 25% leaching and 30% mineralization loss from leaves and needles, Ward 1984). However, the majority of woody debris exhibits a substantially greater degree of decomposition and would likely be processed at rates greater than for sound wood. Therefore, if we assume losses of 5 mm·yr^{-1}, approximately 100 g FPOM·m^{-2}·yr^{-1} would be generated from LWD processing and 375 g·m^{-2}·yr^{-1} from FWD. Together these values are greater than estimates of total litter fall (360 g·m^{-2}·yr^{-1}) and more than 10 times average FPOM standing crop (Naiman and Sedell 1979).

The data of Triska and Cromack (1980) may also be used to estimate quantities of FPOM generated from fine wood. A conservative estimate of FWD processing might be approximately 10%·yr^{-1}. Using this value, FWD processing would generate approximately 100 g FPOM·m^{-2}·yr^{-1} (based on FWD mass from Table 1). Although this estimate is lower than the 375 g·m^{-2}·yr^{-1} cited above for FWD processing, LWD and FWD together could still be a source for 200 g FPOM·m^{-2}·yr^{-1}. This amount is equivalent to quantities of FPOM generated from leaf and needle processing and still many times greater than mean annual FPOM standing crop.

Lignin and Cellulose Content of Particulate Detritus

Lignin is a major component of potential allochthonous inputs and various detrital size fractions in Mack Creek (Table 2). The lignin content of FPOM was approximately 43% AFDW, very similar to that of both soil particulate organic matter and

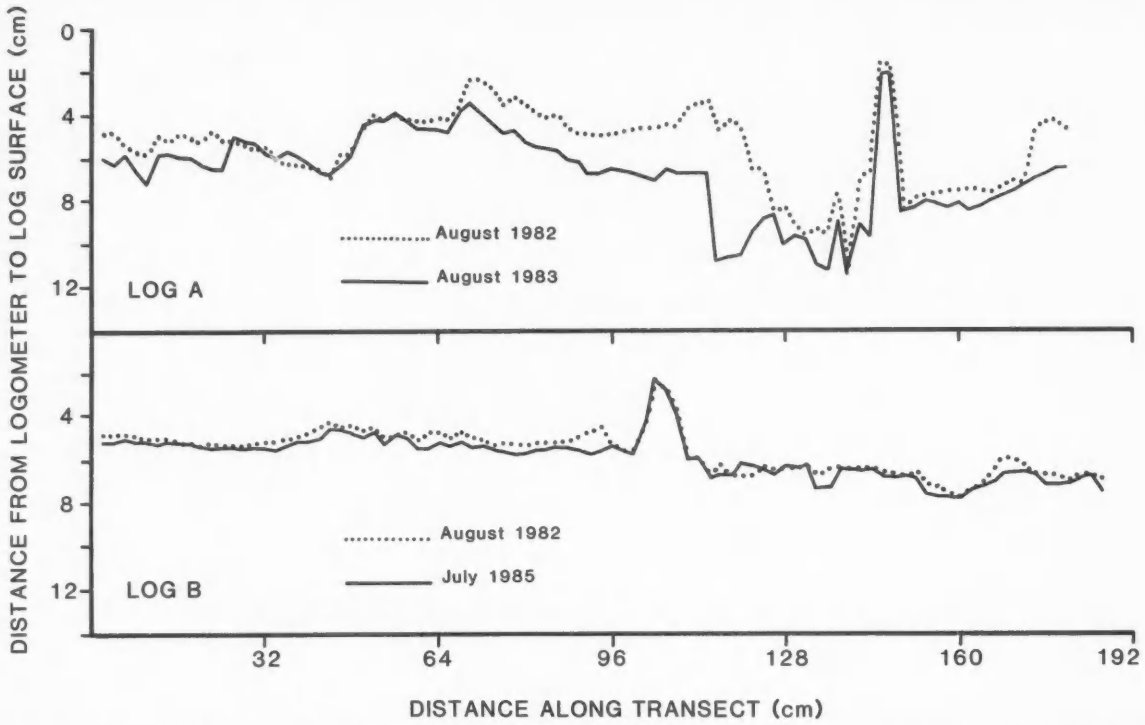

FIG. 4. Changes in the surface profile of two logs in Mack Creek as measured by the logometer. Log A was repositioned by high stream flows in the winter of 1983 and yielded data for only 1 yr. Log B was measured at yearly intervals, but only the first (1982) and last (1985) measurements are presented. Note exaggeration of the vertical scale.

the surfaces of LWD (Table 2). A survey of 27 logs in Mack Creek and its tributary Snag Creek revealed that the average lignin content of surface wood was 43.4% AFDW. A wide range of values was encountered, reflecting variations in both the diversity of decay states among separate pieces of debris as well as within individual logs. Some logs possessed low lignin concentrations (23–30% AFDW) similar to that of living wood, while at the other extreme, well-decayed logs contained up to 87% AFDW lignin. FWD contained almost 60% lignin (Table 2). These smaller sized pieces were primarily cones and twigs, as well as pieces of bark and chips of wood. The larger pieces were usually small branches. The lignin content of soil particulate organic matter was also 43% AFDW, very similar to that of LWD (Table 2).

By comparison, the lignin content of fresh allochthonous inputs is much less. The lignin content of Douglas-fir needles is 25% AFDW, and the content of vine maple (*Acer circinatum*) and red alder (*Alnus rubra*) leaves is near 10% AFDW (Table 2). In general, the lignin content of FWD is substantially greater than that of fresh chips and twigs, and the cellulose content is much reduced (Table 2). Although FPOM and soil were enriched in lignin as compared with leaves and needles, they contained substantially less cellulose than did fresh allochthonous inputs. This likely reflects the continued decomposition of the material once it reaches the FPOM size category.

SEM Observations of FPOM

Samples of FPOM and soil were examined under SEM to determine the extent to which wood particles could be observed. This analysis demonstrated that wood particles were most abundant in the 1–0.5 mm particle size range. However, they were recognizable even in sizes <0.01 mm. Approximately 35% of particles 1–0.5 mm were recognizable as wood, the remainder being insect and leaf parts plus many of unidentifiable origin. Particles 0.25–0.1 mm were 28% wood derived. Percentages of woody particles were less in smaller size classes. Samples from the 0.1–0.053 mm range yielded 15% of particles recognizable as wood. These must be considered conservative estimates, since only particles of obvious wood origin were counted as such.

Discussion

For stream systems where accumulations of coarse and fine woody debris are large, we believe that processing of this debris can produce a significant input to the FPOM pool. The abundance of submerged wood surface area and mass at Mack Creek makes it a site where wood processing could play a particularly significant role. We have based this conclusion not only on the large amounts of wood that were present in the stream, but also because degradation and erosion of wood surfaces were measurable on an annual basis, a high percentage of FPOM particles was clearly of wood origin when viewed with SEM, and the lignin and cellulose content of FPOM was similar to that of surface wood.

Large accumulations of both coarse and fine debris exist in Mack Creek, but much of it lies above winter base flow water

levels. Our estimates of LWD mass were approximately one third of that previously reported for this stream (Anderson et al. 1978; Keller and Swanson 1979); however, ours included only that debris submerged during winter. Substantial amounts of LWD also exist above the winter base flow height, although above 0.4–0.7 m it may contribute little to the FPOM pool. Our estimates of FWD surface area are larger than those of Anderson et al. (1978); however, we included all forms of fine wood >1 cm in diameter, whereas they reported data only for small branch wood.

Even though the results of these studies demonstrate the abundance of wetted wood surface area, our values are undoubtedly underestimates. Surface area calculations assumed smooth surfaces and did not take into account the roughness inherent in natural wood. The surface area of the ends of logs also was not calculated. A more important consideration is that within the 220-m study reach there were no large debris dams. Debris dams serve as retention structures for all types of organic detritus and have been observed to trap massive amounts of both large and fine wood (Bilby and Likens 1980; Cummins 1980; Triska and Cromack 1980). Below our study site on Mack Creek, two such dams occur, each containing wood wetted as the stream flow percolates through the structure. Unfortunately, accumulations of wood associated with these debris dams (some as high as 5–8 m in Mack Creek) were not considered in this study because of the difficulty in measuring buried woody debris in such large structures.

Much of the data presented here are expressed per unit surface area of wood. Although the data could be expressed in units of mass, we believe that the production of FPOM from large wood is best expressed in relation to surface area. The vast majority of wood decomposition occurs in the outer few millimetres of surface (Triska and Cromack 1980; Aumen et al. 1983) and thus, the majority of the wood mass is not metabolically active and not subject to processes which generate FPOM.

The production rate of FPOM is related to the extent of previous microbial degradation and softening of the wood surface. The FPOM is generated when the surface tissue, softened by the differential mineralization of cellulose, becomes sufficiently weakened to be removed by mechanical abrasion from streamflow. Removal of the surface layers exposes the fresh wood substrate beneath, which then can be colonized and degraded to continue the cycle of FPOM production. All of our FPOM production data were derived from Douglas-fir logs. Compared with hardwoods, conifer wood is likely to be processed much slower. Thus, in streams where LWD from hardwoods is abundant, FPOM generation per square metre of wood surface could be greater than in streams where conifers dominate.

At present it is not clear whether LWD or FWD contributes more to the FPOM pool. Fine wood constitutes by far the majority of submerged surface area (Table 1) and in the terrestrial environment is more rapidly processed than coarse wood (Triska and Cromack 1980). However, the majority of FWD is located in more protected areas of the channel, whereas LWD is often oriented more perpendicular to the direction of streamflow and exposed to greater current velocities and to greater erosive forces.

Our calculations suggest that FPOM generated from wood has the potential to be greater than that generated from leaf and needle litter processing. Even if one assumes that all surface wood of all LWD is relatively sound, FPOM inputs could be approximately 50% of that generated from leaf and needle litter processing. However, the surfaces of most logs in Mack Creek are partially decomposed and thus subject to more rapid processing than sound wood. Assuming only a slightly less conservative processing rate, inputs to FPOM would be greater than total annual litter fall. The actual contribution from LWD remains to be determined, for these estimates are not weighted for logs of different decay state, for orientation of the logs (which may affect the erosive capabilities of the current), or for the fact that upstream and downstream sides of logs may be processed at different rates. Our estimates of FPOM production also include that proportion of wood volume lost to mineralization. At present we have no good estimates of mineralization rates, but existing evidence suggests that it is quite slow and is likely small in proportion to fragmentation rates. Turnover rates of large wood based on microbial respiration alone are calculated in terms of centuries. However, few logs older than 75–100 yr are ever observed in Mack Creek (Triska and Cromack 1980). We believe that the difference is due to fragmentation of LWD surfaces.

The production of FPOM from wood surfaces should be greater in winter months than in summer. Rising water levels associated with high winter rainfall increase greatly the amount of submerged woody debris subjected to the physical abrasion of current. The normal winter rise in water level of 0.4 m over summer base flow in Mack Creek increases wetted wood surface by 300%, and during storm flows would of course be even greater. Degradation of wood surfaces continues throughout the year on both submerged and exposed surfaces; however, microbial activity is greater during the summer months (Aumen et al. 1983). Temperatures are warmer, and for many logs exposed by the lower water levels, reductions in water saturation occur, which may enhance diffusion of oxygen through the wood stimulating microbial metabolism. This exposure likely enhances the erodability during winter. There is also likely to be some contribution to the FPOM pool from large wood located above winter base flow. Rotting of this wood, under essentially terrestrial conditions, would produce FPOM as a result of either wind action, rain, alternate freezing and thawing, or processes similar to dry raveling in soils.

An additional source of wood-derived FPOM may be lateral transport from soils in the riparian zone. Riparian soils near Mack Creek contain large amounts of buried wood, and woody debris exists in great abundance on adjacent slopes (Harmon et al. 1986). Therefore, any erosion inputs to the stream channel may contain particles derived from wood. No erosion data exist for Mack Creek, but does for Watershed 10, a second-order stream channel near Mack Creek. Based on data for surface erosion, soil creep, and root throw (Swanson et al. 1982) and from lateral movement of coarse particulate litter (Triska and Cromack 1980), we calculated that erosional inputs contributed 225 g soil organic matter $\cdot m^{-2} \cdot yr^{-1}$. This is somewhat higher than annual litterfall estimates of 151 $g \cdot m^{-2} \cdot yr^{-1}$ (Triska and Cromack 1980) and compares favorably with potential wood-derived FPOM inputs to Mack Creek. The extent to which wood-derived particles are present in soils is not known, but the average lignin content of soil is very near that of FPOM (Table 2). This source may also be important in streams where the quantities of wood in channel storage are small. The overall contribution of soil movement processes to wood-derived FPOM in streams is unknown at this time and warrants further study. Given the potential inputs of FPOM from soil and LWD processing, it appears that leaf and needle

litter inputs can no longer be assumed to constitute the majority of organic matter inputs to a stream system. In many situations, to omit these inputs could result in a serious underestimate of carbon flux into the system.

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Fecundity and Egg Retention of Some Sockeye Salmon (*Oncorhynchus nerka*) Stocks in British Columbia

J. I. Manzer and I. Miki

Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

Manzer, J. I., and I. Miki. 1985. Fecundity and egg retention of some sockeye salmon (*Oncorhynchus nerka*) stocks in British Columbia. Can. J. Fish. Aquat. Sci. 43: 1643–1655.

The fecundity and egg retention of anadromous female sockeye salmon (*Oncorhynchus nerka*) collected during 1971–82 from several stocks in British Columbia undergoing controlled fertilization to enhance adult sockeye production were examined. The relationship between egg number and postorbital–hypural length based on 863 females representing 14 stocks was not consistent between all age-types, stocks, and years, probably because of inadequate sample size in some instances. Combined samples, however, revealed a significant positive relationship between postorbital–hypural length and egg number for age 1.2, 1.3, and 2.2 females. Mean absolute fecundity for the respective age-types was 3218, 4125, and 3544 eggs. For samples of 10 or more females, significant stock and annual differences were detected when individual mean absolute fecundity was adjusted to a postorbital–hypural length of 447 mm, but not for females of different age. A comparison of mean fecundities for coastal stocks with historical data for interior British Columbia stocks suggests that coastal stocks are 18% more fecund than interior stocks. Possible causal mechanisms for this regional difference are hypothesized. Examination of 796 carcasses (representing five stocks) for egg retention revealed a range from totally spawned to totally unspawned females, with 56% of the carcasses containing 20 eggs or less and 68% containing 50 eggs or less. The mean egg retention based on all samples combined was estimated to be 6.5% of the mean individual fecundity. This value was reduced to 3.9% when stock means were averaged.

Les auteurs ont étudié la fécondité et la rétention des œufs chez des femelles de saumon rouge (*Oncorhynchus nerka*) anadromes prélevées de 1971 à 1982 de divers stocks de la Colombie-Britannique faisant l'objet d'une fécondation dirigée visant à accroître la production de saumons rouges adultes. La relation entre le nombre d'œufs et la longueur postorbital–hypural, basée sur 863 femelles de 14 stocks, n'était pas constante pour tous les types d'âge, stocks et années. Cela s'explique probablement par une taille d'échantillon insuffisante dans certains cas. L'ensemble des échantillons révélait cependant une relation positive significative entre la longueur postorbital–hypural et le nombre d'œufs chez les femelles d'âge 1,2, 1,3 et 2,2. La fécondité moyenne absolue pour ces types d'âge respectifs était de 3 218, 4 125 et 3 544 œufs. On a décelé, pour les échantillons de 10 femelles ou plus, des écarts significatifs entre stocks et années lorsque la fécondité moyenne absolue était ajustée à une longueur postorbital–hypural de 447 mm, mais aucun écart n'a été noté entre les femelles d'âges différents. La comparaison des fécondités moyennes des stocks côtiers avec les données historiques de stocks de l'intérieur de la Colombie-Britannique porte à croire que la fécondité des stocks côtiers est de 18 % supérieure à celle des stocks de l'intérieur. Les auteurs proposent des mécanismes pouvant expliquer cet écart entre régions. L'examen de 796 carcasses (cinq stocks) pour la rétention des œufs a révélé l'existence d'une gamme allant d'une ponte totale à l'absence totale de ponte; 56 % des carcasses contenaient 20 œufs ou moins et 68 % en contenaient 50 ou moins. Le taux de rétention moyen des œufs, basé sur l'ensemble des échantillons, a été estimé à 6,5 % de la fécondité individuelle moyenne. Cette valeur était réduite à 3,9 % si l'on faisait la moyenne des moyennes des stocks.

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In 1970, the Pacific Biological Station initiated an experiment in Great Central Lake on Vancouver Island to determine the effects on sockeye salmon (*Oncorhynchus nerka*) production of controlled additions of inorganic nitrogen and phosphorous to an oligotrophic nursery lake (Parsons et al. 1972; LeBrasseur et al. 1978). The total run size and the commercial catch of sockeye produced by brood years associated with lake fertilization increased significantly (Manzer 1976; LeBrasseur et al. 1978). These observed increases led the Canadian Department of Fisheries and Oceans in 1977, in cooperation with the Province of British Columbia as part of the Salmonid Enhancement Program (SEP), to extend controlled fertilization to other nursery lakes in coastal British Columbia in joint effort to increase sockeye production (Stockner et al. 1980). By 1983, 17 lakes supporting anadromous sockeye had been or were being treated, some for as many as 8 yr.

Definitive assessment of response of sockeye salmon to lake fertilization is an important goal of the Lake Enrichment Program (LEP). Data on annual escapements and size of resulting total runs have been used by several investigators, notably Ricker (1954), to develop stock and recruitment models which describe productive capacity of individual stocks of different size. More precise information is potentially available if production indices from egg deposition to various preadult

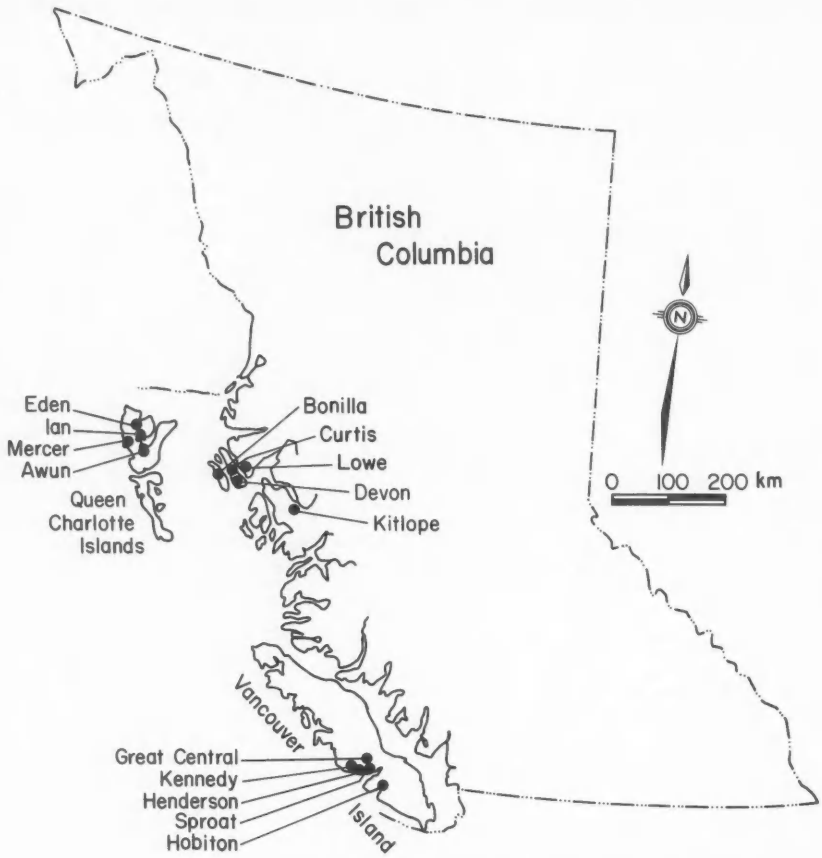

FIG. 1. Location of sockeye salmon stocks sampled during the study.

life stages can be estimated. In this regard, LEP investigators estimate juvenile sockeye production from potential egg deposition. This approach dictates the need for reliable data on fecundity relationships, egg retention, and sex ratios for sockeye utilizing subject lakes.

The purpose of this paper is to report the results of studies on the fecundity and egg retention of female sockeye of enriched lakes. Differences between lakes, years, and age-types are examined. The paper also compares these observations with historical fecundity information reported for other sockeye stocks.

Materials and Methods

Fecundity

During 1971–82, ovaries were collected from 863 sockeye from 14 of the 17 stocks[1] in British Columbia whose nursery lake was selected for enrichment (Fig. 1; Table 1). The maximum number of years for which fecundity data are available for any stock is 5 (Great Central and Henderson), and except for Hobiton (4 yr), all other stocks were sampled for 1, 2, or 3 yr.

Sockeye were caught during migration into nursery lakes by traps set in fishways located on rivers a short distance downstream from the lake outlet or taken on spawning grounds by river seines prior to spawning. Samples representing the Great Central, Sproat, Henderson, and Kennedy Lake stocks prior to 1980 were collected by LEP personnel and subsequently by contract personnel. Samples from northern coastal sockeye stocks were obtained by investigational staff in 1979 and by contract personnel in 1980. Samples of the Queen Charlotte Islands stocks were collected during spawning surveys by local fisheries protection officers. Sampling a relatively large number of stocks scattered throughout coastal British Columbia militated against implementation of a rigid standard sampling scheme common to all stocks in all years. However, comparison of mean postorbital–hypural lengths in fecundity samples and in random samples from the respective escapement indicated that the group mean lengths did not differ significantly in 29 of 30 tests (two-sample t-test). Providing these two sets of samples do not suffer from the same kinds of sampling bias, it can be concluded that females in the fecundity samples were representative of the female population, at least in body length.

Ovaries from Great Central, Sproat, and Henderson lakes sockeye were removed in the laboratory from whole fresh fish

[1]In this paper the term "stock" represents a production system (i.e. lake) and "run" represents the adults returning to that system annually.

TABLE 1. Sockeye salmon stocks sampled for fecundity studies and the years during which samples were obtained.

Stock	Year sampled												Total
	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	
Vancouver Island													
Great Central	×	×	×						×	×			5
Sproat									×	×			2
Henderson						×	×	×	×	×			5
Hobiton									×	×	×	×	4
Kennedy										×			1
Northern Coastal													
Kitlope									×	×			2
Lowe									×	×	×		3
Bonilla										×	×		2
Devon										×			1
Curtis										×	×		2
Queen Charlotte Is.													
Eden									×				1
Ian									×	×			2
Awun									×	×			2
Mercer									×				1

and preserved in Gilson's fluid (Green 1936). Ovaries from sockeye taken in other locations were removed in the field and placed in individual cotton bags, appropriately labelled for individual identification, placed in plastic containers containing 10% formalin solution, and shipped to the Pacific Biological Station for subsequent processing. Age of individual sockeye was usually determined from scales and otoliths, if both materials were available, or from otoliths only if prespawning resorption rendered the scales useless for determining total age. Preparation of scales and otoliths for age determination is outlined in Manzer (1976). The European system for age designation of salmonids was used in this study (e.g. 1.2 designates a salmonid that spent one winter in freshwater and two winters in the ocean before returning to spawn). Postorbital-hypural length was recorded for each sockeye, since this measurement is minimally affected by dimorphic changes associated with maturation and spawning.

The fecundity of individual females prior to 1980 was determined volumetrically and thereafter by visual counts of eggs. With each method, egg skeins for individual females were first broken into clusters and individual eggs were teased from surrounding ovarian tissue, care being taken not to break or lose any eggs. The counting method was considered to be accurate. The volumetric method involved counting the eggs in a subsample of the ovary, placing these eggs in a graduated cylinder with water measuring their volume by water displacement, and then extrapolating the subsample egg number to the displacement volume of the remaining separated eggs. This was done either for left and right ovaries separately, and summed to obtain the total egg number for the individual female, or by combining the separated eggs from both ovaries prior to volumetric determinations. The variance ratio test was used to test the hypothesis that egg numbers derived by the respective methods did not differ significantly. Test data were obtained from 26 females whose eggs, from either a single ovary or both, were counted and then estimated volumetrically.

Fecundity of salmonids has been shown to be positively related to size and age (Bagenal 1978; Rounsefell 1957) and can differ significantly between stocks (Rounsefell 1957; Foerster 1968; Healey 1978; Beacham 1982; Healey and Heard 1984). Variability in fecundity due to size differences among females was minimized by adjusting individual fecundity to a postorbital–hypural length of 447 mm, the grand mean length of the 863 females in the combined samples. Standardization to grand mean length used the formula

$$F_a = F_o \frac{\bar{L}^b}{L}$$

where F_a = adjusted fecundity of individual, F_o = observed fecundity of individual, $\bar{L}$ = mean postorbital–hypural length of all individuals, L = observed length of individual, and b = regression coefficient of log F_o on log L. The variables were transformed to common logarithms. Differences between adjusted fecundity among years, systems, and age-types were tested by using ANOVA (BMDPIV in Dixon 1981) on samples arbitrarily selected to contain 10 or more individuals. Regression statistics were calculated only for individual samples containing three or more females, although all females sampled were included in calculations for combined ages.

The mean fecundity of stocks represented by samples containing 10 or more individuals was compared with the means for other British Columbia stocks reported in the literature or in the archives at the Pacific Biological Station. The mean fork length (FL) for the latter stocks was converted to postorbital–hypural length (PO–HL) by using the regression equation PO–HL (mm) = 21.4685 + 0.7861FL (mm) developed by Bilton (1970) from length measurement data for Babine female sockeye. The mean number of eggs per centimetre postorbital–hypural length was calculated and this value was adjusted to a standard PO–HL = 447 mm.

Egg Retention

Data on egg retention were collected during 1981–83 from 796 sockeye carcasses from runs to Great Central, Sproat, Henderson, Bonilla, and Devon lakes during stock enumeration and sampling surveys after spawning had virtually ceased. Carcasses were collected from shallow nearshore spawning areas in lakes or from gravel bars or pools on spawning streams. The body cavity of each carcass was thoroughly examined for unspawned eggs, and those present were counted and

TABLE 2. Summary of observed fecundity and length (mm) data and regression statistics (based on common logarithms) for sockeye stocks sampled. $*P \leq 0.05$; $**P \leq 0.01$. Presentation of similar statistics for individual year and age-types by stock would make this table prohibitively long. Data are available from the archives at the Pacific Biological Station, Nanaimo, B.C.

Stock	Year	Age[a]	Sample size	Fecundity		Length		Regression statistics		
				Mean	SE	Mean	SE	*a*	*b*	*r*
Great Central	1971	1.2, 1.3, 2.2	12	3555	196.1	497.6	15.90	4.1600	−0.2290	0.1293
	1972	1.2, 1.3, 2.2	48	3662	107.6	428.9	4.93	−1.7460	2.0150	0.7857**
	1973	1.2, 1.3, 2.2	85	3835	85.8	447.9	4.27	−0.6380	1.5901	0.6938**
	1979	1.2, 1.3, 2.2	82	3309	59.9	428.3	3.01	−0.1358	1.3873	0.5210**
	1980	1.2, 1.3, 2.2	118	3251	50.6	431.4	2.90	−0.9291	1.6839	0.6978**
	All years	1.2	244	3240	32.8	422.5	1.41	−0.5583	1.5479	0.5047**
		1.3	66	4278	83.6	492.5	3.42	3.6481	−0.0082	0.0029
		2.2	35	3613	106.4	430.5	5.54	1.6850	0.7091	0.3135
		Total	345	3476	37.2	436.7	1.97	−0.3464	1.4702	0.6179**
Sproat	1979	1.2, 1.3	54	3175	91.7	450.9	5.13	−2.1358	2.1275	0.7931**
	1980	1.2, 1.3, 2.2	38	3017	101.7	434.1	6.30	−1.4365	1.8614	0.7607**
	All years	1.2	61	2889	72.9	429.6	3.48	−2.8950	2.4114	0.7543**
		1.3	21	3786	114.6	496.4	5.15	−2.0616	2.0908	0.6667**
		2.2	10	3032	130.5	421.5	8.57	2.0092	0.5597	0.2456
		Total	92	3110	68.4	444.0	4.05	−1.7326	1.9712	0.7795**
Henderson	1976	1.2, 1.3	32	3945	173.2	464.8	4.25	−2.9611	2.4542	0.6201**
	1977	1.2, 1.3	18	4998	245.7	464.7	8.65	−1.3686	1.8971	0.6710**
	1978	1.3	18	5735	176.0	500.0	2.94	−1.9567	2.1165	0.4260
	1979	1.2, 1.3, 2.2	31	3878	136.3	431.9	6.64	−3.3520	2.6313	0.7927**
	1980	1.2, 1.3	27	4083	128.8	443.8	7.46	−0.2334	1.4508	0.7338**
	All years	1.2	72	3835	101.2	435.1	3.47	−0.9022	1.6968	0.5199**
		1.3	50	5142	126.1	490.6	2.63	−2.1796	2.1861	0.4690**
		2.2	4	4163	279.7	438.2	12.38	−2.5455	2.3330	0.9846**
		Total	126	4364	95.2	457.2	3.31	−2.0713	2.1432	0.7080**
Kennedy	1980	1.2, 1.3, 2.2	20	3944	118.2	458.7	7.87	4.103	−0.1924	0.1029
Hobiton	1979	1,2 1.3	42	2854	85.2	427.7	6.03	−0.4985	1.5009	0.7049**
	1980	1.2, 1.3	19	2927	122.8	436.6	6.25	−1.0598	1.7148	0.5577*
	1981	1.2, 1.3	23	2670	84.5	425.9	6.95	0.7902	1.0013	0.4800*
	1982	1.2, 1.3	37	2591	67.9	422.3	6.10	0.3701	1.1579	0.6480**
	All years	1.2	89	2636	47.8	411.8	2.52	−0.5946	1.5339	0.5233**
		1.3	31	3107	85.7	471.3	4.803	−0.5527	1.5116	0.5464**
		2.2	1	2722		415.0				
		Total	121	2757	45.3	427.1	3.24	−0.1755	1.3728	0.6296**
Kitlope	1979	1.2, 1.3, 2.2	16	4348	142.0	483.1	6.88	2.80	0.3115	0.1303
	1980	1.2, 1.3, 2.2	12	4227	223.4	490.8	9.44	−1.4930	1.9005	0.7048*
	All years	1.2	11	4288	173.7	472.7	4.92	2.4226	0.4509	0.1079
		1.3	8	4641	225.1	522.5	6.48	0.3215	1.2295	0.3205
		2.2	9	4001	224.1	471.1	7.35	0.0965	1.3098	0.3810
		Total	28	4296	123.4	486.4	5.58	0.7065	1.0877	0.4262*
Lowe	1979	1.2, 1.3, 2.2	10	3760	256.1	529.5	14.91	−2.7870	2.3333	0.8967**
	1980	1.3	13	3941	156.4	563.5	5.55	6.8771	−1.1946	0.2844
	1981	1.3	2	3807		530.0				
	All years	1.2	3	3435	384.3	495.0	22.55	−2.4497	2.2200	0.9008
		1.3	20	4018	118.4	561.2	4.77	3.1972	0.1467	0.0409
		2.2	2	2887		485.0				
		Total	25	3858	128.5	547.2	7.30	−0.6215	1.5350	0.5868**
Eden	1979	1.2, 2.2	5	4176	214.2	430.6	5.05	0.1407	1.3204	0.3042
Ian	1979	1.2, 1.3, 2.2	13	3025	164.8	428.5	4.88	−2.4415	2.2475	0.4784
	1980	1.2, 1.3	13	3279	211.8	467.7	10.12	−0.3907	1.4595	0.4747
	All years	1.2	14	3047	180.4	425.8	5.04	−5.0631	3.2476	0.6649**
		1.3	11	3293	221.0	478.6	8.15	−1.2632	1.7805	0.4294
		2.2	1	3069		495.0				
		Total	26	3152	134.0	448.1	6.76	−0.1321	1.3663	0.4722*
Awun	1979	1.2, 1.3	12	3542	179.6	468.3	8.5	−2.2254	2.1760	0.7820**
	1980	1.2, 1.3	15	3039	130.6	461.0	9.1	0.8862	0.9730	0.4360
	All years	1.2	12	2990	127.6	427.2	6.2	−1.7540	1.9867	0.6609*
		1.3	15	3481	165.6	477.9	5.3	3.7909	−0.0959	0.0203
		Total	27	3262	116.4	455.4	6.3	0.3751	1.1783	0.4481*

TABLE 2. (*Concluded*)

Stock	Year	Age[a]	Sample size	Fecundity Mean	Fecundity SE	Length Mean	Length SE	Regression statistics a	b	r
Curtis	1980	1.2, 1.3	4	3458	153.7	458.7	3.8	11.1743	−2.8693	0.5330
	1981	1.2, 1.3, 2.2	11	4138	173.4	489.5	9.5	1.6390	0.7342	0.3782
	All years	1.2	3	3601	135.8	438.3	16.7	1.7456	0.6854	0.7138
		1.3	11	3998	192.5	492.3	6.2	−0.7681	1.6216	0.4460
		2.2	1	4561		490.0				
		Total	15	3956	153.5	481.3	7.8	0.6841	1.0848	0.4919
Devon	1980	2.2	4	3386	104.9	435.0	3.54	11.4263	−2.9931	0.783
Bonilla	1980	1.3, 2.2	3	3398	523.4	476.7	26.80	0.2510	1.2218	0.4742
	1981	1.2, 1.3, 2.2	22	3490	114.9	464.3	5.98	1.7605	0.6666	0.2483
	All years	1.2	14	3420	139.3	452.9	3.62	−4.3063	2.9504	0.5696*
		1.3	7	3604	303.9	507.1	5.65	−3.2740	2.5216	0.3140
		2.2	4	3467	126.6	438.7	4.73	−5.0255	3.2415	0.9328*
		Total	25	3479	113.7	465.8	5.95	1.5437	0.7469	0.2822
Mercer	1979	1.3, 2.2	4	2825	248.3	462.5	12.50	−4.3150	2.9127	0.9043
All stocks	All years	1.2	538	3218	30.0	425.4	1.16	−1.6830	1.9706	0.5775**
		1.3	247	4125	59.7	495.1	2.05	1.0629	0.9411	0.2618**
		2.2	78	3544	74.4	437.6	3.49	1.3418	0.8335	0.3151**
		Total	863	3507	29.6	447.1	1.43	−0.6669	1.5855	0.6033**
All stocks (samples ≥10)	All years	1.2	497	3186	30.6	424.7	1.15	−1.6771	1.9681	0.5644**
		1.3	147	4301	77.8	493.5	2.58	1.2059	0.8978	0.2514**
		2.2	30	3510	128.0	426.7	5.80	0.3224	1.2232	0.4580*
		Total	674	3444	33.7	439.7	1.52	−1.2899	1.8223	0.6485**

[a]The European system is used to designate age. The numeral preceding the decimal represents the number of winters in freshwater and the numeral following the decimal represents the number of winters in the ocean.

TABLE 3. Frequency of direction and significance ($P \leq 0.05$) of regression coefficient for fecundity − postorbital−hypural length relationship for sockeye, by sample size.

Sample size	Fecundity − postorbital−hypural length b value: + slope, Significant	+ slope, Not significant	− slope, Not significant
<10	1	18	9
10−20	7	10	3
21−30	4	1	0
30+	5	0	0
Total	17	29	12

the number recorded. If the unspawned eggs were numerous, or if a female had died totally unspawned, this condition was noted and the eggs were placed in appropriately labelled cotton bags and then stored in plastic buckets containing 10% formalin solution until counted in the laboratory. Size and age of each carcass were determined by the methods employed in fecundity determinations.

Except for dead females with intact egg skeins, it is impossible to know precisely what proportion of the total eggs the retained eggs constititute. In this study, incidence of egg retention of varying magnitude was determined from frequency distribution data as well as from regression analysis of relationships of fecundity and egg retention on size of female (independent variable). Differences in these relationships for different stocks and age-types were investigated using analysis of covariance (BMDP1V), with length (millimetres) the covariate. Of the 796 carcasses examined, 13 were judged to have died prior to release of any eggs and were omitted from any additional analysis of egg retention. The actual number of unspawned eggs for each female was increased by one egg so as to include in the analyses those females which had released all their eggs. The variates were transformed to common logarithms. Egg retention was expressed as a percent of the mean estimated fecundity.

Results

Fecundity

The hypothesis of no difference between actual and estimated (volumetric) egg numbers in the test sample of 26 females could not be rejected ($P(|F_{25,25}|\,1.007 > 0.50)$). The mean estimated egg number was greater than the actual number by 20 eggs, or 1.1%. This difference was considered insufficiently large to warrant adjustment of the volumetric estimates of individual fecundity.

The mean length of age 1.2, 1.3, and 2.2 female sockeye from all stocks and years sampled ranged from 412 to 495, 471 to 561, and 415 to 495 mm, and the range in estimated fecundity of the respective age and size groups was 2636−4288, 3107−5142, and 2722−4561 (Table 2). Slopes of the regression of log fecundity on log length ranged from 0.451 to 3.248 for 1.2 females, −0.096 to 2.522 for 1.3 females, −2.993 to 3.242 for 2.2 females. The mean length of all (863) females examined was 447 mm and the mean fecundity was 3507. The slope of regression of fecundity on length was 1.586.

A significant positive relationship was found between fecundity and postorbital−hypural length for ages 1.2, 1.3, and 2.2

TABLE 4. Fecundity of sockeye salmon adjusted to postorbital−hypural length of 447 mm by stock, year, and age-type (limited to sample size ≥10).

Stock	Year	Age	Treated[a]	No. in sample	Mean adjusted fecundity	SE FEC[b]
Great Central	1972	1.2	N	22	3697	117.6
		1.3	N	16	4256	106.7
		2.2	N	10	3496	103.8
	1973	1.2	Y	51	3670	66.0
		1.3	N	24	3605	113.0
		2.2	Y	10	4198	229.1
	1979	1.2	N	72	3460	57.9
	1980	1.2	Y	94	3463	42.4
		1.3	N	15	3760	88.9
Sproat	1979	1.2	N	39	3136	78.3
		1.3	N	15	2895	55.3
	1980	1.2	N	22	3210	66.6
		2.2	N	10	3135	135.1
Henderson	1976	1.2	N	20	3467	153.5
		1.3	N	12	3684	176.6
	1977	1.2	N	13	4538	227.5
	1978	1.3	N	18	4521	121.8
	1979	1.2	Y	25	4323	108.7
	1980	1.2	Y	14	4343	113.2
		1.3	Y	13	4484	147.5
Kennedy	1980	1.2	N	12	3876	152.5
Hobiton	1979	1.2	N	32	3007	72.3
		1.3	N	10	2959	103.7
	1980	1.2	Y	14	3308	131.5
	1981	1.2	Y	18	2938	105.5
	1982	1.2	Y	25	2646	58.1
		1.3	Y	11	2734	115.6
Lowe	1980	1.3	N	13	5188	199.0
Ian	1979	1.2	N	10	3614	196.0
Bonilla	1981	1.2	N	14	3285	110.1

[a]Treated lake conditions during lacustrine development of juvenile sockeye. Y = yes, N = no.
[b]Standard error of adjusted fecundity.

and for these ages combined ($P < 0.01$) (Table 2). For all samples combined, approximately 36% of the variability in egg numbers is associated with change in fish length. This value was increased to 42% when analysis was limited to samples of ≥10 females. Table 3 summarizes the individual regression coefficients for stock and age-type in different years according to direction and frequency, by sample size. Of 58 data sets, regression coefficients were positive for 46 sets and negative for 12. Of the data sets with positive slopes, 17 were significant ($P < 0.05$). The relatively low incidence (29.3%) of data sets with a significant positive fecundity−length relationship is believed to be a function of inadequate sample size rather than a biological phenomenon which influences egg number.

Mean fecundities (± 2 SE) of females adjusted to 447 mm postorbital−hypural length are presented in Table 4 by age-type for several stocks represented by samples of 10 or more individuals. ANOVA tests on three stocks (Great Central, Henderson, Hobiton) having either two or three age-types indicated significant annual variability in fecundity of females of the same age-type in six of seven trials (Table 5). Considering these stocks but without reference to age-type, annual differences in mean fecundity for a female measuring PO−HL = 447 mm ranged from 225 to 1071 eggs, or differences ranging between 6.5 and 30.9%. Moreover, for Great Central and Hobiton age 1.2 females, mean fecundity tended to be lower in later years for which samples were available. The opposite trend is suggested for Henderson Lake age 1.2 and 1.3 sockeye. Analysis of interage differences in fecundity of Great Central, Sproat, and Hobiton stocks indicated no significant difference in mean egg numbers between females of different age-type but similar size in three of four trials (Table 6).

Although fecundity data for a particular age-type are not available for all stocks for the same year, some major stock differences are apparent. For example, for age 1.2 sockeye, Henderson Lake females on average were significantly ($P < 0.05$) more fecund (4167) than Great Central (3572), Sproat (3173), or Hobiton (2974) females (Table 2). A similar ranking over approximately the same range of mean fecundities is noted among stocks for age 1.3 females. Age 1.3 Lowe Lake females were the most fecund group examined, although data were available for only 1980.

Mean adjusted fecundity for the various stocks listed in Table 4 and for other British Columbia sockeye stocks reported by earlier investigators (Foerster and Pritchard 1941; Aro and Broadhead 1950; Ward 1952; Bilton 1970) or available from unpublished reports in the archives of the Pacific Biological Station (Appendix) is given in Fig. 2. The mean fecundity values for earlier studies were calculated from regression equations, adjusted to 447-mm PO−HL. Regression equations for some stocks in earlier studies are not age specific, but

TABLE 5. Results of analysis of variance testing annual variability in fecundity of sockeye of standard postorbital–hypural length of 447 mm, within stock by age (sample size ≥10). *$P = 0.05$; **$P < 0.01$.

Stock	Age	Years	Degrees of freedom	F
Great Central	1.2	1972, 1973, 1979, 1980	3, 234	6.77**
	1.3	1972, 1973, 1980	2, 51	7.55**
	2.2	1972, 1973	1, 17	5.50*
Henderson	1.2	1976, 1977, 1979, 1980	3, 68	6.27**
	1.3	1976, 1978, 1980	2, 39	6.87**
Hobiton	1.2	1979, 1980, 1981, 1982	3, 85	8.63**
	1.3	1979, 1982	1, 18	1.97

TABLE 6. Results of analysis of variance testing variability in fecundity of sockeye of different age and standard postorbital–hypural length of 447 mm, within stock by year (sample size ≥10). *$P < 0.05$.

Stock	Year	Ages	Degrees of freedom	F
Great Central	1973	1.2, 1.3, 2.2	2, 81	3.52*
	1980	1.2, 1.3	1, 106	0.17
Sproat	1980	1.2, 1.3	1, 29	3.51
Hobiton	1982	1.2, 1.3	1, 33	0.57

this should not preclude their use, since the present study as well as others on the fecundity of other species of Pacific salmon (Beacham 1982) have shown that differences in fecundity among age-classes are not significant once differences in size of females have been accounted for. A zonal trend in the adjusted mean fecundity appears to exist among the various stocks in British Columbia. In general, coastal stocks including those examined on the Queen Charlotte Islands and on Vancouver Island are 18% ((3585 − 3027) ÷ 3027 = 0.18) more fecund that interior British Columbia stocks. This difference was found to be statistically significant ($t = 4.56$, $P < 0.001$) when mean fecundities for individual stocks and years for the respective regions were compared.

Fecundity did not appear to be influenced significantly by duration of marine residence according to egg counts available for age 1.2 and age 1.3 sockeye for Great Central, Henderson, and Hobiton stocks and representing collectively 5 brood yr (Table 7). The largest observed difference between mean counts for 2- and 3-ocean fish was 300 eggs (1975 brood for Great Central Lake) and is just barely significant at $P = 0.05$.

Data are insufficient to demonstrate whether nursery lake enrichment had any effect on fecundity of sockeye. Mean adjusted fecundities of age 1.2 females returning to Great Central Lake in 1972 (3697, SE = 118) and 1979 (3460, SE = 58) which did not experience lake treatment did not differ significantly from mean fecundities of females returning in 1973 (3670, SE = 66), a run which experienced lake treatment (Table 4). For Henderson Lake females the results obtained are inconsistent. The mean adjusted fecundity of untreated age 1.2 females (3467, SE = 153) which returned in 1976 was significantly less than that for females returning in 1977 (4538, SE = 227). The mean fecundity of the latter group, however, did not differ significantly from the mean for females returning in 1979 (4323, SE = 109) and 1980 (4343, SE = 113), which as juveniles experienced treated conditions. These kinds of differences can also be noted for age 1.3 females, although because of the age difference the data are for different brood years. For age 1.2 Hobiton females the maximal difference in mean fecundities of 662 eggs between treated broods (1980, 1981, 1982) exceeded that (343) between the only untreated brood (1979) and the treated broods. The mean fecundity of untreated age 1.3 females (2959, SE = 104) returning in 1979 did not differ significantly from treated females (2734, SE = 116) returning in 1982, although evidence is scant because sample sizes were relatively small.

Egg Retention

The number of eggs found in dead females ranged from nil to totally intact ovaries, according to examination of a total of 796 carcasses from five stocks (Great Central, Sproat, Henderson, Devon, Bonilla) in 1 or more yr. Correlation analysis using nonparametric tests (Spearman rank on Great Central and Sproat; Kendall coefficient of concordance on Henderson) indicated significant association between ranks of numerical groupings of eggs retained in different years by individual stocks, thus permitting pooling of data for individual stocks with more than 1 yr of data. The data for individual stocks, whether for 1 or more yr combined, indicate that a high proportion of females released all or most of their eggs during spawning and that the number which retained relatively large numbers of eggs progressively diminished (Table 8). This relationship becomes more apparent when the data for all stocks and years are combined. Excluding 13 females (1.6%) which died without releasing any eggs, 56% of the carcasses contained ≤20 eggs, 68% contained ≤50 eggs, and females with ≥100 eggs amounted to 4% of the samples.

Examination of pooled data for stock differences suggests that the distribution observed for the Henderson stock departed somewhat from the general pattern indicated by the data in Table 8. Females with <20 eggs constituted the largest group for the stock but a significant number of females died with higher egg numbers than was the case for other stocks examined. The phenomenon is particularly obvious from samples collected in 1982 and 1983 and is suggested for 1981. A comparison of ranks of egg retention (Spearman rank correlation) categories for Henderson Lake samples with those for non-Henderson stocks indicated that the group rankings differed significantly ($r_s = 0.57$, $P < 0.05$). It should be noted that Henderson Lake samples included lakehead spawners as well as those spawning in Clemens Creek, a 6.5-km stream above the lake which is subject to variable water flows, particularly during the fall and winter months. Egg retention was higher in samples from Clemens Creek than those from beach spawning areas in 1982 and 1983, the 2 yr for which there are data, and significantly so ($P < 0.05$) when the samples in the 2 yr

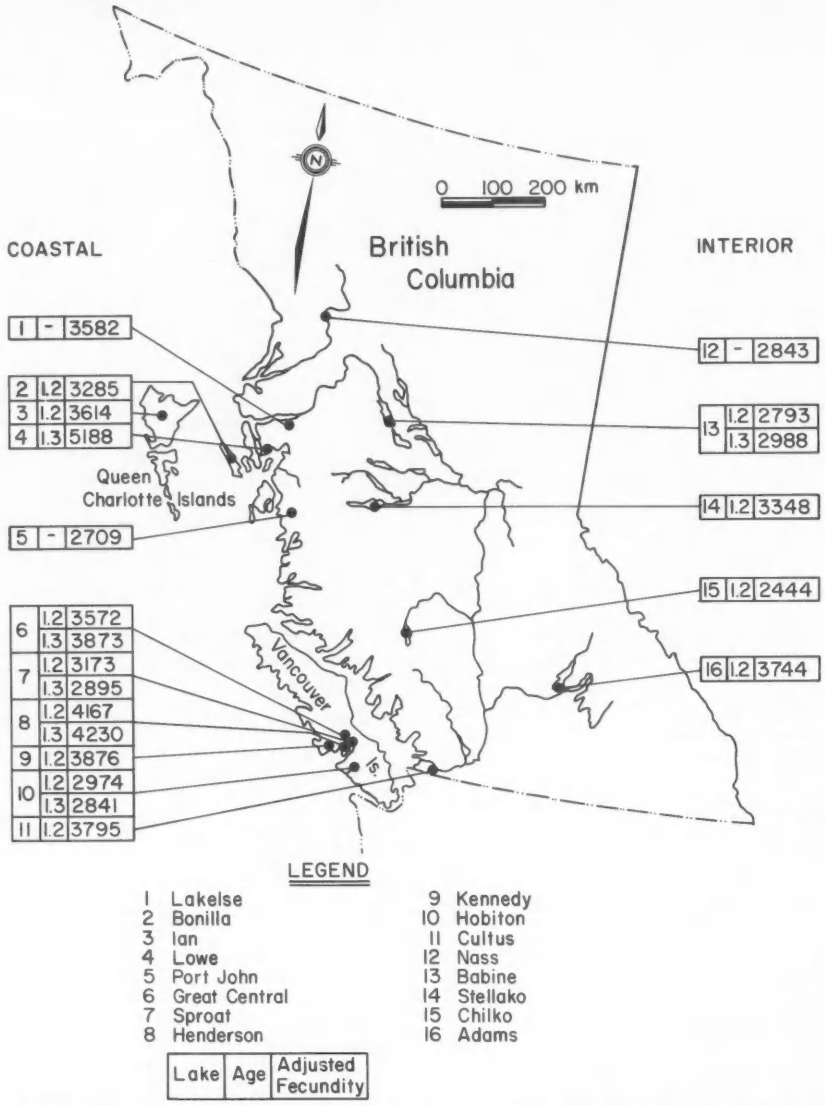

FIG. 2. Fecundity of coastal and interior stocks of sockeye salmon in British Columbia. Fecundity is adjusted to female sockeye of 447 mm (postorbital–hypural length) using data given in Table 3 and Appendix.

TABLE 7. Egg numbers for 1.2 and 1.3 sockeye from the same brood year (SE in parentheses). Egg number is for sockeye adjusted to 447 mm postorbital–hypural length (Table 4). $*P < 0.05$.

Stock	Brood year	Egg number	
		Age 1.2	Age 1.3
Great Central	1968	3697 (±118)	3605 (±113)
	1975	3460 (±58)	3760 (±89)*
Henderson	1973	4538 (±227)	4521 (±122)
	1975	4323 (±109)	4484 (±147)
Hobiton	1977	2938 (±105)	2739 (±116)

were combined (Table 9). The difference between the two spawning areas is manifested in the combined Henderson stock samples and probably accounts for the difference noted between Henderson and other stocks.

Egg retention samples for all stocks in all years were not sufficiently large to permit examination for annual variation by age, and accordingly all years were pooled. Considering combined ages, the number of unspawned eggs tended to be weakly but positively correlated with size of female. Of the five stocks examined, a significant relationship ($P \leq 0.05$) was observed only for Great Central and Henderson stocks (Table 10). When individual age-types represented by 10 or more carcasses were examined, a significant positive relationship between egg re-

TABLE 8. Frequency distribution for egg retention in female sockeye salmon, by stock and year. Correlation coefficient: $r_s = 0.97$ (Spearman rank, $P < 0.01$); $r_s = 0.90$ (Spearman rank, $P < 0.01$); $W = 0.75$ (Kendall coefficient of concordance, $P < 0.05$).

	Great Central			Sproat			Henderson				Devon	Bonilla	All data	
No. of eggs	1981	1983	Total	1981	1983	Total	1981	1982	1983	Total	1981	1981	Total	%[a]
0–20	60	52	112	53	32	85	20	78	63	161	48	32	438	55.9
21–50	22	7	29	18	11	29	10	9	16	35	1	4	98	12.5
51–100	16	5	21	12	1	13	8	7	8	23	5	3	65	8.3
101–200	6	2	8	7	5	12	1	11	8	20	2	2	44	5.6
201–500	7	2	9	9	1	10	6	23	20	49	1	2	71	9.1
501–1000	3	0	3	3	0	3	1	8	18	27	0	2	35	4.5
1001–3000	1	0	1	0	0	0	3	12	9	24	0	5	30	3.8
>3000	0	0	0	0	0	0	0	0	0	0	0	2	2	0.3
Unspawned	2	0	2	0	0	0	1	2	8	11	0	0	13	—
Total	117	68	185	102	50	152	50	150	150	350	57	52	796	100.0

[a]Based on data that excludes 13 unspawned dead females.

TABLE 9. Summary of egg retention in female sockeye carcasses collected from spawning grounds in Henderson Lake and Clemens Creek, 1982 and 1983.

	1982		1983		Total	
Location	No.	Mean (SE)	No.	Mean (SE)	No.	Mean (SE)
Lakehead	49	125.3 (43.5)	47	129.1 (52.3)	96	128.2 (33.7)
Clemens Creek	98	303.0 (53.9)	95	354.1 (58.2)	193	328.9 (39.6)

TABLE 10. Regression estimators for relationship between number of eggs retained and postorbital–hypural length (mm) for female sockeye by stock and age, years combined. Regression statistics are derived from common logarithms. Number of eggs retained by each female was increased by 1 egg. $*P = 0.05$; $**P = 0.01$.

			Egg retention		Length		Regression statistics		
Stock	Age	Sample size	Mean	SE	Mean	SE	*a*	*b*	*r*
Great Central	1.2	112	55.2	21.10	414.1	1.96	1.6869	−0.0288	0.008
	1.3	65	67.0	15.05	466.2	2.83	−15.0344	6.1089	0.167
	2.2	6	44.3	25.67	405.8	9.78	−17.0744	7.0862	0.399
	All	183	59.0	13.97	432.3	2.44	−10.0452	4.2176	0.176*
Sproat	1.2	106	40.1	6.85	406.2	1.78	6.5499	−2.0800	0.060
	1.3	46	108.0	28.01	461.5	3.72	9.4187	−2.9938	0.090
	All	152	60.3	9.94	423.2	2.65	−4.8388	2.3068	0.104
Henderson	1.2	45	227.3	70.22	426.7	2.60	−14.0075	5.8963	0.109
	1.3	294	255.1	28.85	478.4	1.02	−20.2416	8.1377	0.135*
	All	339	251.5	26.67	471.5	1.35	−10.3143	4.4404	0.109*
Devon	1.2	9	25.0	15.47	436.1	6.11	−102.8580	39.2036	0.809**
	1.3	23	15.2	5.31	481.5	4.64	−12.0870	4.7808	0.143
	2.2	3	71.7	64.18	461.7	7.27	50.3810	−18.3944	0.260
	All[a]	57	20.1	5.90	473.8	3.44	−4.934	2.102	0.077
Bonilla	1.2	21	118.1	57.30	449.0	5.48	18.9977	−6.7873	−0.162
	1.3	21	429.9	186.73	479.5	5.00	−17.2068	6.9382	0.124
	2.2	5	2.0	2.0	453.0	8.60	25.4435	−9.5020	−0.375
	All[a]	52	326.6	104.8	464.8	3.83	2.912	−0.636	−0.085

[a]Includes all age-types in addition to those shown.

tention and size of female was found only for Henderson Lake age 1.3 females ($P < 0.05$). However, it should be noted that egg retention was significantly related to size ($P < 0.01$), for age 1.2 Devon Lake females, according to a sample of nine individuals.

Egg retention was not consistently significantly related to size of female. Also, some females retained very large numbers of eggs. Accordingly, it seemed reasonable to calculate egg retention for individual stocks from stock mean egg retention expressed as a percent of the estimated mean fecundity (Table 2) for that stock, rather than from predicted egg retention values derived from regression analysis. The results of these calculations, based on values given in Tables 2 and 10, are presented in Table 11. Obviously, on average, the number of eggs produced by a female and not released at spawning is minor, regardless of age of female. Mean egg retention ranged

TABLE 11. Percent egg retention in sampled British Columbia sockeye stocks.

Stock	Age	No.	Mean fecundity[a]	No.	Mean egg retention[b]	% egg retention
Great Central	1.2	244	3240	112	55	1.7
	1.3	66	4278	65	67	1.6
	2.2	35	3613	6	44	1.2
	All	345	3476	183	59	1.7
Sproat	1.2	61	2889	106	40	1.4
	1.3	21	3786	46	108	2.8
	All	92	3110	152	60	1.9
Henderson	1.2	72	3835	45	227	5.9
	1.3	50	5142	294	255	5.0
	All	126	4364	339	252	5.8
Devon	All	4	3386	57	20	0.6
Bonilla	1.2	14	3420	21	118	3.5
	1.3	7	3604	21	429	11.9
	2.2	4	3467	5	2	0.1
	All	25	3479	52	326	9.4
Stock, ages combined		863	3507	783	229	6.5

[a]Data from Table 3.
[b]Data from Table 11.

between 0.1 and 11.9%. The estimated grand mean based on all observations combined is 6.5%. This value may be inflated, since it was based on some small samples with relatively high values (e.g. Bonilla Lake). The mean of stock means is 3.9%. Egg release is equally successful for all age-types for which samples are sufficiently large, but the possibility of within- and between-stock differences is suggested when data for Henderson sockeye are compared with those for other stocks. Mean egg retention for Great Central and Sproat stocks was just under 2%, while that for Henderson sockeye was 5.8%, or almost three times as great.

Discussion

Positive relationships between egg number and length (postorbital−hypural) of sockeye salmon was observed in 37 of 58 data sets representing different age-types for several stocks in British Columbia. Positive significant relationships ($P < 0.01$) were obtained for each age-type examined after combining samples representing different stocks and years. These findings agree with those made on sockeye stocks in Alaska (Rounsefell 1957; Pella and Jaenicke 1978) and in British Columbia (Withler 1950; Foerster and Pritchard 1941; Aro and Broadhead 1950), as well as for other species of *Oncorhynchus* (Rounsefell 1957; Beacham 1982). In the present study after combining samples of ≥10 females, 42% of the variability in egg number was accounted for by length of female. This level of association is stronger than the 31−32% found by Rounsefell (1957), using data for Cultus Lake (British Columbia) sockeye presented by Foerster and Pritchard (1941). A comparison of the length−fecundity relationships in this and Rounsefell's study must be made with reservation, since Rounsefell's results were based on untransformed data. Regardless of the manner in which the data were handled, it is clear that more than one half of the variability in fecundity is associated with factor(s) other than length of female. In this study, small sample size for some stocks and/or years would appear to be an important factor influencing results.

Mean estimated fecundity of sockeye salmon was found to vary between age-types, stocks, and years. In general, age 1.3 sockeye were the most fecund (4125) followed by age 2.2 (3544) and age 1.2 (3218) sockeye, reflecting a positive relationship with body size (495, 438, and 426 mm, respectively). Departures from this general relationship are probably the result of inadequate sample size. However, even after adjusting fecundity to a standard length, significant stock and annual differences were still indicated, but for a given run fecundity at different ages did not differ greatly.

Rounsefell (1957) compared fecundity of salmonids, including sockeye salmon, along the Pacific Coast of North America and suggested that southern stocks were more fecund than northern stocks. He ascribed this apparent clinal tendency to differences in growth rates, the southern stocks being faster growing and maturing at younger ages. Considering adjusted fecundities, results of the present study show considerable latitudinal irregularity in fecundity of British Columbia stocks, and when examined in relation to data presented in the literature do not fully support Rousenfell's observation. For chinook salmon (*O. tshawytscha*), Healey and Heard (1984) suggested that if fecundity varies with latitude, the trend is for greater fecundity at higher latitudes. A similar tendency was observed for chum salmon (*O. keta*) (Beacham 1982). However, the present study deals with stocks over a considerably reduced latitudinal range and as a result, local variability may obscure the broad picture. For example, Hobiton sockeye at the southern end of Vancouver Island are considerably less fecund (<3000 eggs) than Ian (3614) and Lowe (5188) sockeye salmon. Of considerable interest, however, is the deduction that females from coastal stocks are an estimated 18% more fecund than those of similar size from interior stocks. Considering that data for the interior stocks used in the comparison were collected mainly during the 1940's and that during much of the time since then (1951−75) the average size of sockeye in British Columbia has declined (Ricker 1981), it may be argued that differences in fecundity between coastal and interior stocks for a similar period may be larger than what appears

at first glance. Data obtained during the present study are insufficient to positively explain the apparent zonal differences in stock fecundity, if indeed real. It may be hypothesized that these differences represent adaptive mechanisms for maintenance of stocks utilizing markedly different environments. To elaborate, coastal lakes are extremely unstable as a result of winter discharge and are mainly oligotrophic (Stockner and Shortreed 1979; Stockner et al. 1980). Consequently, smolts from these lakes are generally small compared with most interior lake smolts (1–2 vs. 4–6 g) (Hyatt and Stockner 1985). Since marine survival of smolts is positively related to size (Ricker 1962) in general, coastal smolts presumably experience lower marine survival rates than interior lake smolts. For coastal stocks to maintain populations under these conditions, high egg production is advantageous. Alternatively, interior lakes are more stable and productive, but stocks utilizing these lakes are faced with longer and more arduous migration routes than coastal stocks. Somatic material which otherwise could be directed into egg production is expended as physical energy. Fecundity data for the Fraser River stocks provide some support for this alternative hypothesis. Mean stock fecundity in order of decreasing magnitude is Cultus (3795), Adams (3744), Stellako (3348), and Chilko (2444). Considering various physical aspects (migration distance, water velocity, elevation, etc.) which conceivably affect migration, conditions appear to be least demanding of Cultus sockeye and most demanding of Chilko sockeye.

Irrespective of the degree to which productivity and accessibility of nursery lakes may have on population fecundity, the controlled additions of inorganic nutrients in proper amounts and ratios have potential for increasing the fecundity of treated stocks. Sufficient evidence now exists that smolts originating from treated nursery lakes on average are heavier (larger) than those originating from untreated lakes (Hyatt and Stockner 1985), through improved production at the primary and secondary trophic levels. Larger smolts may be translated into faster marine growth and consequently earlier maturation. Positive correlations between food levels and egg production in salmonids have been reported by Scott (1962) and Bagenal (1978). Rounsefell (1957) found for 5-yr-old sockeye of similar freshwater life history in Karluk Lake, Alaska, that 2-ocean-winter fish were significantly more fecund than 3-ocean-winter fish. Experiments specifically designed to investigate interrelationships between stock fecundity, food supply, sockeye salmon growth, as well as other factors, could be informative.

This study indicates that a very high proportion of spawning females release all but a very few of their eggs. Approximately two thirds (68%) of the carcasses examined had 50 or less eggs. A very small proportion died with a large number of eggs, and some with complete ovaries. The observations on almost complete expression of eggs during spawning and prior to death agree with Foerster's (1968) studies and his summary of findings of other researchers.

Egg retention was greater in river spawners than lake spawners, according to observation on Henderson sockeye in 1982 and 1983. The limited nature of the data precludes positive identification of the causal mechanisms, and their relative influence. It is possible, however, that high spawner density may have induced higher egg retention in Clemens Creek spawners: in 1982 and 1983 the estimated available area per female was 20 and 25 ft^2, respectively, which from visual observations appeared to be less than the area available to lake spawners. Increased egg retention in relation to spawner density has been observed for pen-retained sockeye (Mathisen 1962) and pink salmon (*O. gorbuscha*) (Hanavan and Skud 1954) and in chum salmon spawning in controlled-flow conditions (Lister and Walker 1966; Schroder 1973). It is also possible that contrasting physical conditions associated with river and lake spawning areas could have influenced egg retention. In 1982 and 1983, peak spawning times (late October) coincided with high river levels and strong water velocities caused by heavy rains. Under these physically demanding conditions, females may have altered spawning behavioural activities by transferring energy from egg expression to holding and defending redd sites. Schroder (1973) observed for chum salmon that the occurrence of agnostic behaviour increased with increasing density of spawners.

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Appendix

TABLE A.1. Fecundity of British Columbia sockeye salmon indicated by sources other than the present study (egg number adjusted to postorbital–hypural (PO–HL) length of 447 mm).

Stock	Year	No. in sample	Age	Mean fecundity	Mean PO–HL[a] (cm)	Adjusted fecundity	Source
Nass[b]	1934	35	—	3461	54.4	2843	Rounsefell 1957
Babine[b]	1946	59	—	3281	50.0	2933	Withler 1950
	1947	73	—	3187	48.6	2931	Withler 1950
	1949	57	—	3353	49.1	3053	Withler 1950
	1962	49	1.2	2750	43.8	2806	Bilton 1970
		79	1.3	3378	49.9	3026	Bilton 1970
	1963	81	1.2	2793	43.2	2892	Bilton 1970
		47	1.3	3387	49.9	3035	Bilton 1970
	1964	43	1.2	2607	42.5	2740	Bilton 1970
		133	1.3	3207	48.2	2972	Bilton 1970
	1965	55	1.2	2645	42.8	2762	Bilton 1970
		107	1.3	2997	46.2	2901	Bilton 1970
	1966	59	1.2	2644	42.8	2762	Bilton 1970
		111	1.3	3285	48.9	3004	Bilton 1970
	Mean (1962–66)	287	1.2	2688	43.0	2793	
		477	1.3	3251	48.6	2988	
Lakelse[c]	1939	24	—	3888	48.2	3606	Aro and Broadhead 1950
	1948	22	—	3860	48.5	3558	Aro and Broadhead 1950
	1949	41	—	3699	47.8	3459	Aro and Broadhead 1950
	1953	28	—	4079	49.2	3706	P.B.S. archives
	Mean	115				3582	
Port John[c]	1949	18	—	2425	42.5	2552	P.B.S. archives
	1950	9	—	2157	42.0	2298	P.B.S. archives
	1951	5	—	2632	44.8	2626	P.B.S. archives
	1953	8	—	2809	45.2	2776	P.B.S. archives
	1954	9	—	2711	45.5	2801	P.B.S. archives
	1955	15	—	2577	43.0	2857	P.B.S. archives
	1956	15	—	2626	43.3	2709	P.B.S. archives
	1958	14	—	2998	43.9	3053	P.B.S. archives
	Mean	93				2709	
Chilko[b]	1945	20	1.2	2652	47.7	2485	Ward 1952
	1946	24	1.2	2442	47.0	2322	Ward 1952
	1948	100	1.2	2682	47.5	2525	Ward 1952
	Mean	144				2444	
Stellako[b]	1950	98	1.2	3347	48.8	3066	Ward 1952
	1951	50	1.2	4206	51.8	3630	Ward 1952
	Mean	148				3348	
Adams[b] River	1950	79	1.2	4048	50.5	3583	Ward 1952
	1951	39	1.2	4457	51.0	3906	Ward 1952
	Mean	118				3744	

TABLE A.1. (*Concluded*)

Stock	Year	No. in sample	Age	Mean fecundity	Mean PO−HL[a] (cm)	Adjusted fecundity	Source
Cultus[c]	1932	46	1.2	4310	48.1	4005	Foerster and Pritchard 1941
	1933	47	1.2	3796	46.5	3649	Foerster and Pritchard 1941
	1934	75	1.2	4282	48.5	3946	Foerster and Pritchard 1941
	1935	55	1.2	4067	48.5	3748	Foerster and Pritchard 1941
	1937	35	1.2	3864	46.2	3738	Foerster and Pritchard 1941
	1938	47	1.2	4012	48.1	3728	Foerster and Pritchard 1941
	1943	56	1.2	3723	45.5	3657	Ward 1952
	1944	40	1.2	4103	47.2	3886	Ward 1952
	Mean	401				3795	

[a]Calculated from PO−HL (mm) = 21.4685 + 0.7861 = FL (mm) (Bilton 1970).
[b]Interior stock.
[c]Coastal stock.

NOTES

Fitting and Comparing Several Growth Curves of the Generalized von Bertalanffy Type

R. K. Misra

Department of Fisheries and Oceans, Fisheries Research Branch, P.O. Box 550, Halifax, N.S. B3J 2S7

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The earlier version of the von Bertalanffy growth formula (VBGF) has three parameters and is a special case of the "generalized" VBGF which has four parameters. The special VBGF can easily lead to erroneous results whereas the large flexibility built into the generalized VBGF enables it to provide a biologically realistic fit to the data. I present a statistical method and a computer program in Fortran (1) to fit the generalized VBGF to a sample (a sample comprises several age groups, with one or more observations in an age group), (2) to examine statistically the fit to a sample, and (3) to compare statistically these curves (and their individual parameters) fitted to several samples. An example is provided, which also compares the fit of the generalized VBGF with the special VBGF.

La version antérieure de l'équation de croissance de von Bertalanffy (VBGF) renferme trois paramètres et représente un cas spécial de la formule « généralisée », qui possède quatre paramètres. La VBGF spéciale peut facilement conduire à des résultats erronnés, alors que le haut degré de flexibilité introduit dans la VBGF généralisée permet à cette dernière une adaptation biologiquement réaliste aux données. Nous présentons ici une méthode statistique et un programme machine en Fortran dans le but 1) d'adapter la VBGF généralisée à un échantillon (un échantillon est constitué de plusieurs groupes d'âge, chaque groupe étant l'objet d'une ou de plusieurs observations), 2) de faire l'analyse statistique de l'adaptation à un échantillon et 3) de comparer ces courbes statistiquement (et leurs paramètres individuels) adaptées à plusieurs échantillons. Nous donnons un exemple dans lequel nous comparons également la VBGF généralisée à la VBGF spéciale.

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The study of growth is of fundamental importance in theoretical and applied ecology and is central to fishery biology. The von Bertalanffy growth formula (VBGF) (von Bertalanffy 1938, 1960) is used as a model for animal growth and has been employed in the general theory of fishing since the work of Beverton and Holt (1957). Pauly (1980) employed the VBGF to develop a new approach to estimating natural mortality which is a key parameter in fish population dynamics. The VBGF also provides an empirical description of growth, and its usefulness in intra- and inter-population (stocks, races, subspecies, and species) comparisons is widely recognized (e.g. see Brousseau 1979; Craig 1980; De Veen 1976; Francis 1981; Goldspink 1979; Holleman and Dieterich 1978; Kingsley 1979; Kohlhorst et al. 1980; Laws et al. 1975; Pauly 1978, 1979; Pitt 1967; Ricker 1975; Ricklefs 1968; Saunders and Powell 1979). "Comparisons of the parameters of growth curves affords a general and flexible technique for comparing populations in different areas, or the same population at different times or under different conditions" (Kingsley 1979).

The VBGF is also a mechanistic model, developed as a "physiological" formula for growth. Pauly (1979) reviewed its mathematical formulation and properties (see also Alagaraja and Jhingaran 1976; Taylor 1962). These are stated briefly as follows. 1. Let s and w denote fish body surface and weight, respectively. The basic equation for the rate of growth, dw/dt, is

$$(1) \quad dw/dt = \mathrm{Hs} - \mathrm{Kw}$$

where Hs and Kw express anabolism and catabolism, respectively. 2. Let s and w increase proportionately with the powers of a and b of fish size (length), where $b > a$. The surface factor D $(=b - a)$ can be computed from

$$(2) \quad D = 3(1 - d)$$

where d is an exponent relating the growth of gills and oxygen consumption with weight. The value of d may be obtained from metabolic or gill studies (see also Muir 1969). Pauly (1979) proposed an empirical equation to estimate d, and other methods are also suggested (e.g. see Alagaraja and Jhingaran 1976). Integration of equation (1) leads to what Pauly (1979) called the "generalized VBGF," and for the special case of isometric growth when $d = 2/3$, giving $D = 1$, it leads to the

"special VBGF." Knowledge of the generalized VBGF goes back as far as Richards (1959) (see also Chapman 1961).

Problems pertaining to the special VBGF, which severely restrict its usefulness, were recognized and parameters of the generalized VBGF were discussed and interpreted by several authors (e.g. Alagaraja and Jhingaran 1976; Pauly 1979). Some of these are as follows: 1. The assumption $D = 1$ is often erroneous. The value of d varies frequently among groups of fish, in the range $2/3-1$ (so that D varies in the range $0-1$) although this range may be extended (i.e. d can be $<2/3$ or >1). 2. The more erroneous is the assumption $D = 1$, the greater will be the difference between Y_{max} and α, where Y_{max} is the maximum size attained by fishes in a given environment and α (equations (3) and (4) given later in the text) is the asymptotic (theoretical maximum) size. This can lead to an erroneous fit of the special VBGF. An important aspect of the relationship between α and Y_{max} is the effect of the length of the life span. The value of α for the fish growing under the special VBGF could far exceed the Y_{max} value. Pauly (1979) cited a study on the growth of the giant bluefin (*Thunnus thynnus*) where the fit of the special VBGF was good statistically, yet unrealistic biologically, as α (=505 cm) was much larger than Y_{max} (≅330 cm). The real value of D was 0.3, indicating that the proper function to fit was the generalized VBGF. The generalized VBGF estimated the asymptotic value of Y as 332 cm which was close to its maximum value. 3. The parameter t_0 (equation (4) given later in the text) is of little, if any, biological significance, as fish do not grow in the manner described by the VBGF. The parameter K (equation (4)) has greater physiological significance. A precise estimate of K will yield a better approximation to the growth pattern and can be obtained in conjunction with D. It has been referred to as a "catabolic coefficient" by Alagaraja and Jhingaran (1976) and as a "stress factor" by Pauly (1979). Growth performance and asymptotic size of fish are highest when K and the associated stress are lowest.

In view of the importance of the generalized VBGF, it is obvious that there exists a need, addressed in this note, for developing a statistical method and a computer program (1) to fit the generalized VBGF to a sample (a sample comprises several age groups, with one or more observations in an age group), (2) to examine statistically the fit to a sample, and (3) to compare statistically these curves (and their individual parameters) fitted to several samples. In choosing such a statistical method to fit a VBGF and to compare fitted curves, the following are noted: 1. The standard deviation of length varies with age. This was shown for the cod data used by Misra (1980) for the special VBGF and in the numerical example for the generalized VBGF given in this presentation. 2. Heterogeneity of variances is caused by unequal frequencies of age groups (Steel and Torrie 1960, p. 181), which frequently occur in real data. When there is heterogeneity of variances, (i) inefficient estimators of model parameters will result if an unweighted procedure of fitting the equation is employed (Press 1972, Chap. 8) and (ii) Hotelling's T^2 (Bernard 1981, 1982) procedure will not compare two fitted curves adequately (Harris 1975, p. 85; Misra and Smith 1982) except for the uncommon case when samples are large and equal in size.

Statistical Methods and the Computer Program

The generalized VBGF can be expressed in a number of forms. In this presentation the following two forms, which are extensions of those employed for the special VBGF in Misra (1980), are considered:

$$(3)\quad Y_t^D = \alpha^D + \beta^D \cdot \rho^{Dt}$$

$$(4)\quad Y_t^D = \alpha^D\,(1 - e^{KD(t-t_0)})$$

where Y_t is the length at age t, α is the asymptotic Y, K is the Brody growth coefficient (Ricker 1975, p. 221), ρ ($=e^{-K}$) is Ford's growth coefficient (Ricker 1975, p. 222), and t_0 is the hypothetical age at which $Y_t = 0$ if fish had always grown in the manner described in this equation. The computer program was written (in Fortran) bearing in mind that the user may prefer a procedure which first assigns a value to D and then fits the generalized VBGF rather than a procedure where all four parameters are computed simultaneously, for the following reasons: 1. The user may have determined the value of D independently (such as from metabolic or gill studies or by using an empirical equation, as stated earlier in the text). 2. The user may employ a trial and error procedure (e.g., Taylor 1962) to choose a value of D arbitrarily from within a range of values predefined (by the user) by variables named DMAX and DMIN in the program. Fitting of the generalized VBGF to Y-data can be accomplished by any procedure which fits the special VBGF to Y_1-data where $Y_1 = Y^D$ (Pauly 1979, 1984). For weight data, where Y_t is the weight at age t, values of $(\sqrt[3]{Y_t})^D$ may be used (instead of $(\sqrt[3]{Y_t})$) to fit the generalized VBGF. Following Armitage (1971), Bliss (1970), and Williams (1967), Misra (1980) developed a statistical method and a computer program to fit the special VBGF to a sample and to compare several fitted curves and their parameters. This was extended to develop the statistical procedure and the computer program for the generalized VBGF. A detailed account of the program and access to it will be provided in Misra (1986).

The computer program begins by processing samples individually. In fitting the generalized VBGF to a sample, the program starts with the trial value of D for DMAX. If the criteria for an acceptable fit of Y_1 values to the VBGF are not met, DMAX is reduced by STEP (also predefined by the user) and the entire computational procedure is repeated. This process continues until DMAX is reduced to DMIN. If the user wishes to employ a specific value of D (rather than search for a value from within a range), he assigns this value to DMAX and DMIN. It is noted that the specific value of $D = 1$ would fit the special VBGF. For each value of D in equation (3) the transformed Y_1 ($=Y^D$) is fitted the same way as equation (1) of Misra (1980) for untransformed Y. For the transformed data, given an initial estimate r of ρ (which is estimated in the program), the expected asymptotic regression (equation (3)) becomes linear in Y_1 and Z, where $Z = r^t$. An iterative procedure is then employed to improve this estimate for a maximum likelihood (ML) solution. This is done by supplementing, at each iteration, the provisional metameter Z with its auxiliary variate Z_1 ($=tr^{t-1}$). This leads to an estimate $a + bz + b_1z_1$ for a group mean in the multiple linear regression (MLR) of group means of Y_1 on Z and Z_1 (Bliss 1970). In the MLR analysis the user has the choice of using an unweighted or a weighted regression procedure. If age groups differ in variability, a weighted procedure will be desirable, with the user having the choice of assuming whether or not group variances follow a pattern.

If two (or more) values of D yield acceptable fits of equation (3), the D which corresponds to the best fit of the equation is chosen. Several criteria to compare fits are given in Klein (1974, Chap. 6). The program is written in such a way that any

TABLE 1. Estimates of $Y_{1\infty}$, ρ, slope b, and auxiliary slope b_1, their SE and 95% confidence limits and ANOVA of the MLR (sample 1).

Constant	Value	SE	Confidence limits	
			Lower	Upper
$Y_{1\infty}$	1.5939	0.00103	1.5917	1.5960
b	−0.3036	0.00189	−0.3075	−0.2996
b_1	0	0.00074	−0.0015	0.0015
ρ	0.8259	0.00245	0.8208	0.8310

Source	ANOVA of the MLR		
	df	MS	F
All slopes (b and b_1)	2	0.354428	4048.9
Slope (b)	1	0.402448	4597.5
Auxiliary (b_1)	1	0	
Error	21	0.000088	

TABLE 2. Estimates of K, t_0, and $Y_{1\infty}$ and their SE values, by Allen's algorithm (sample 1).

Constant	Value	SE
K	0.1944	0.00102
t_0	−8.4994	0.05704
$Y_{1\infty}$	1.5927	0.00037

TABLE 3. Age group frequencies, observed and expected group means, and their differences for the Y_1 ($=Y^D$; Y in cm; $D = 0.1$) data (sample 1).

Age	Group size	Observed length	Expected length	Difference
3	80	1.4283	1.4228	0.0054
4	357	1.4506	1.4526	−0.0020
5	596	1.4759	1.4772	
6	490	1.4994	1.4975	0.0019
7	302	1.5168	1.5143	0.0025
8	192	1.5303	1.5281	0.0022
9	145	1.5361	1.5396	−0.0034
10	82	1.5444	1.5490	−0.0047
11	83	1.5497	1.5568	−0.0071
12	55	1.5648	1.5633	
13	71	1.5688	1.5686	
14	54	1.5760	1.5730	0.0030
15	51	1.5825	1.5766	0.0059
16	33	1.5796	1.5796	
17	12	1.5848	1.5821	0.0026
18	14	1.5844	1.5842	
19	13	1.5822	1.5859	−0.0037
20	7	1.5944	1.5872	0.0071
21	11	1.5818	1.5884	−0.0066
22	3	1.5706	1.5894	−0.0188
23	3	1.5784	1.5901	−0.0117
25	1	1.5648	1.5913	−0.0266
26	1	1.5817	1.5918	−0.0101
27	1	1.6086	1.5921	0.0165

one of these can be employed with a slight modification of the statements. In the example presented here, the fit with the largest R^2 was chosen as the best fit.

The output for each sample includes the following. 1. A table showing, for each age-group, its frequency, mean Y, mean square (MS), and degrees of freedom (df). 2. A plot of group mean against age. 3. a plot of group MS against age. In fish populations, this plot may be a parabola. A subroutine tests the significance of the parabolic fit by an analysis of variance (ANOVA) procedure and estimates constants of the parabola. 4. For the Y_1 data, (i) an ANOVA of MLR to test the combined effect of Z and Z_1, primary effects of Z and Z_1, and the effect of Z_1 given Z (Misra 1980), (ii) standard errors (SE) and confidence intervals for the constants of MLR and for ρ (Misra 1980), (iii) an unbiased estimate of the total variation in $\bar{Y}_1$, explained by MLR, based on R^2 (Misra 1980), (iv) observed and expected means of Y_1 at each time point employing the MLR equation (differences between these two mean values at each time point is tested for significance (Misra 1980)), and (v) constants α, K, and t_0. The methodology of Allen (1966), commonly used to estimate these constants and their SE values, was used here. In employing Allen's procedure the user may be faced with the difficulty of providing an adequate initial value for K. This difficulty does not exist in the program, as it automatically computes the initial K, as explained in Misra (1980).

If the generalized VBGF curves for various samples differ in their D values, the program terminates at that point. When D values are all equal, samples are compared by χ^2 and by ANOVA (Misra 1980) for Y_1 data. The chi-square procedure compares samples for $Y_{1\infty}$, β, and ρ values employing reciprocals of variances as weights. The ANOVA procedure compares samples for differences in the fits of the entire equation and in individual slopes b and b_1 of the MLR.

An Example

To facilitate comparison of the two VBGF's, the analysis for the generalized VBGF is employed on the same data used for the special VBGF (Misra 1980). Two samples of Atlantic cod (*Gadus morhua*) were collected at the Newfoundland Biological Station in 1968 and 1940 and were designated samples 1 and 2, respectively, in Misra (1980). Length-at-age for 2657 specimens of sample 1 and 385 specimens of sample 2 was analyzed using the generalized VBGF in this presentation and using the special VBGF in Misra (1980). Since the two samples were processed the same way, output for only one (sample 1) is given. In that output, the parts which are not common to the output reported in Misra (1980) and which are an essential part of the output are shown. Complete output may be obtained from the author.

Misra (1980) is referred to for the following: (i) group sizes, their mean Y, MS, and df (table 1); (ii) the plot of group mean Y versus age (fig. 1); (iii) the plot of MS versus age (fig. 2); and (iv) the analysis of the parabola fitted to MS values (table 2). It was noted that the parabola described the fit of the MS values well. This presentation contains the following. Best value of D was 0.1. For the Y_1 data, (a) Table 1 gives estimates of $Y_{1\infty}$, ρ, and slope b and auxiliary slope b_1 of the MLR, their SE values, and 95% lower and upper confidence limits, and ANOVA of the MLR; (b) Table 2 gives estimates of the parameters K, t_0, and $Y_{1\infty}$ and their SE values; (c) Table 3 gives age group frequencies, observed group mean lengths calculated from equation (3), and their differences when significant at the 5% level.

The nonsignificance of the b_1 term, high significance of the combined slopes, and high R^2 (=99.73%) indicate that the generalized VBGF fitted sample 1 very well. The analysis

for sample 2 (with $D = 0.2$) yielded similar results. The program terminated at this point without comparing the generalized VBGF fitted to the two samples (as their D values were different).

The R^2 criterion was employed to compare the fit of the generalized VBGF with the special VBGF. The analysis for the special VBGF presented by Misra (1980) yielded high values of R^2: 99.21 and 94.39% for samples 1 and 2, respectively, indicating that these are not the best data to demonstrate the improvement in fit brought upon by the generalized VBGF. Nevertheless, the better fit of the generalized VBGF (over the special VBGF) to the data of each sample is indicated in the following. 1. The generalized VBGF yielded even higher values (than the special VBGF) of R^2: 99.73 and 96.84% for samples 1 and 2, respectively. 2. An examination of the difference between observed and expected mean lengths showed that the generalized VBGF fitted the "problem" region (the upper end of the data) much better than the special VBGF. To illustrate this, absolute values of difference for age 21 or more (table 5 of Misra (1980) shows that differences were conspicuously large for these age groups) were expressed as percents of the corresponding observed mean length values given in table 5 of Misra (1980) for the special VBGF and in Table 3 of this presentation for the generalized VBGF. For sample 1, these were 6.9 (0.4), 16.0 (1.2), 11.4 (0.7), 23.4 (1.7), 11.5 (0.6), and 5.3 (1.0). Figures within parentheses correspond to the generalized VBGF and those without parentheses to the special VBGF. These show that the generalized VBGF consistently fitted better than the special VBGF.

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Simple Method of Measuring Seston Respiration in Oligotrophic Lakes

R. J. Cornett[1] and F. H. Rigler[2]

Biology Department, McGill University, 1205 Avenue Docteur Penfield, Montreal, Que. H3A 1B1

Cornett, R. J., and F. H. Rigler. 1986. Simple method of measuring seston respiration in oligotrophic lakes. Can. J. Fish. Aquat. Sci. 43: 1660–1663.

A simple technique for measuring the oxygen consumption of seston in oligotrophic waters was developed and tested. Water samples were filtered through 0.45-μm filters. The filters were suspended in biological oxygen demand bottles containing lake water. NaN_3 added to one bottle stopped respiration and served as a control to eliminate abiotic influences during the experiments. The difference in oxygen concentration between the respiring and control samples increased linearly over the 1-d experiments. Filtration of hypolimnetic water samples did not significantly alter the rate of oxygen consumption of the seston. Concentrating the seston increased the change in oxygen concentration so that respiration rates from 2 to 80 mg $O_2 \cdot m^{-3} \cdot d^{-1}$ could be measured. This method is simple, precise, and can measure very low rates of respiration directly.

Nous avons mis au point et testé une méthode simple pour déterminer la consommation d'oxygène du seston dans des eaux oligotrophes. Les échantillons d'eau ont été filtrés à travers des filtres de 0,45 μm. Ces derniers ont été suspendus dans des bouteilles de mesure de la demande biologique en oxygène contenant de l'eau de lac. Une addition de NaN_3 à une bouteille a causé un arrêt de la respiration et a servi de témoin pour éliminer les influences abiotiques au cours des essais. Sur la période d'une journée qu'ont duré les essais, la différence de concentration d'oxygène entre les échantillons avec respiration et les échantillons témoins a augmenté linéairement. La filtration des échantillons hypolimnétiques n'a pas modifié de façon significative le taux de consommation d'oxygène du seston. La concentration de ce dernier a eu pour effet d'accentuer le changement de concentration d'oxygène et de permettre la mesure de taux de respiration allant de 2 à 80 mg $\cdot m^{-3} \cdot j^{-1}$ de O_2. Cette méthode est simple, précise et permet de mesurer directement des taux de respiration très bas.

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Many studies suggest that nutrients are rapidly recycled within the water column of lakes (Rigler 1956; Kajak et al. 1972; Wetzel et al. 1972; Bloesch 1974; Bloesch et al. 1977). However, few hypotheses concerning the respiration of detrital substances have been tested and there are very few generalizations about planktonic respiration (DePinto 1978; Forsberg 1985; Linsey and Lasenby 1985). Often, available methods for studying decomposition have not adequately estimated in situ rates of metabolism (Cole and Likens 1979). Field studies employing single labelled substrates (Wright and Hobbie 1966) are very difficult to compare with other metabolic rate measurements, and laboratory studies alter in situ conditions (Jewell and McCarty 1971). Even the addition of compounds which were naturally labelled under in situ conditions (Saunders 1972; Cole and Likens 1979) can alter the concentrations of metabolites present in the water.

In this note we present and test an alternative method of measuring rates of decomposition in the water column of lakes. Although this method will probably be applicable in a diversity of systems, we have focused our investigations upon the hypolimnion of oligotrophic lakes. These systems are among the most difficult to study because the rates of oxygen consumption are extremely low (Cornett 1982; Cornett and Rigler 1984; R. J. Cornett and F. H. Rigler, unpubl. data).

Methods

The method developed to measure respiration consisted of four steps: sterilization of the containers; collection of water samples; filtering of subsamples and addition of the particulate material; and measurement of the oxygen concentrations.

Before an experiment was conducted, all sampling bottles (60-mL biological oxygen demand, hereafter BOD) were sterilized by autoclaving. The bottles were then kept frozen until just before the water samples were collected.

Water samples were collected from within the hypolimnion using a 4-L VanDorn sampler or a peristaltic pump. Tschumi et al. (1977) demonstrated that the order in which oxygen bottles were filled from a sampler affected the oxygen concentrations of the samples. A simple solution to this potential problem was to fill duplicate bottles simultaneously by splitting the water flowing out of the sampler into two bottles using a "Y" joint. This procedure was used for all experiments. During the filling of the samples, temperature changes were minimized by filling the bottles rapidly, out of direct sunlight, in a large bath of water collected from the same depth as the samples. As the bottles were filled, the water samples were filtered through a 250-μm screen to eliminate all larger zooplankton. Water overflowed each oxygen bottle (>200 mL) to ensure that the samples had minimal contact with the atmosphere. Immedi-

[1]Present address: Atomic Energy of Canada Limited, Research Company, Chalk River Nuclear Laboratories, Chalk River, Ont. K0J 1J0.

[2]Deceased.

TABLE 1. Comparison of control oxygen concentrations ($mg \cdot m^{-3}$) and respiration rates ($mg \cdot m^{-3} \cdot d^{-1}$) of samples that received 0 or 400 μL of NaN_3 (set B) with samples that received 200 μL of NaN_3 (set A). N is the number of pairs in the experiment and SE is the standard error of the mean difference; t statistics calculated to test the hypotheses that the means of the treatments are different are not significant ($p > 0.05$).

			Volume of NaN_3 added			
			Set A	Set B		
Test	Comparison	N	200 μL	0 μL	400 μL	SE
1	Control O_2	12	4072	4074	—	4.6
2	Respiration	6	11.4	11.4	—	3.5
3	Control O_2	12	9254	—	9249	11.2
	Respiration	14	22.6	—	24.8	2.8

ately after filling two pairs of bottles, these samples were returned to the cooler in a bath of water from the same depth. The entire process of filling the bottles raised the temperature of the samples by less than 4° above the in situ temperature (4−13°C). An additional 2 L of water was collected from the same depth and was placed in a plastic bottle rinsed twice with sample water. This sample was also placed in the incubator.

At the lake shore, four subsamples of 250 or 500 mL of water were filtered through a 47-mm-diameter, 0.45-μm membrane filter. The filtration pressure was between 4 and 5 psi (1 psi = 6.895 kPa), and the filtration of each sample was stopped just before the filter was dry. To filter 500 mL of sample required 5−8 min; 250-mL samples were filtered in 2−5 min. During the filtration, aluminum foil shielded the samples from the sun. Each of the four filters was rolled with forceps and dropped into one of the four sample bottles collected from the same depth. To one sample of each pair, 200 μL of 0.8 N NaN_3 was added to stop all respiration. Then the bottles were quickly returned to the incubator. Since each sample was treated identically, the sample with the azide acted both as an estimate of the initial concentration of oxygen in the water and as a control to correct for any changes in oxygen concentrations that were induced by the experimental manipulations.

After 24 h, the samples were removed from the incubator and 200 μL of azide solution was added to the respiring samples. The filters were removed with forceps, Winkler reagents added, and oxygen concentrations analyzed by the methods of Carpenter (1965) and Bryan et al. (1976). The rate of respiration was calculated as

$$(1) \quad R = \frac{1000 \cdot V \cdot (C - F)}{D \cdot (V + \mathrm{VF})}$$

where V is the volume of the sample bottle, VF is the volume of sample filtered, and C and F are the oxygen concentrations (milligrams per litre) measured in the control sample and the respiring sample. The respiration rates (R) are expressed as milligrams oxygen per cubic metre per day by dividing the change in concentration by the duration of the experiment (D, in days).

The utility of the method outlined depends upon evidence that the treatment does not influence the results, that the experiments are reproducible, and that the resulting respiration rates can be compared with other components of the carbon cycle. A detailed comparison of the results of this method and rates of oxygen depletion will be made later (R. J. Cornett and

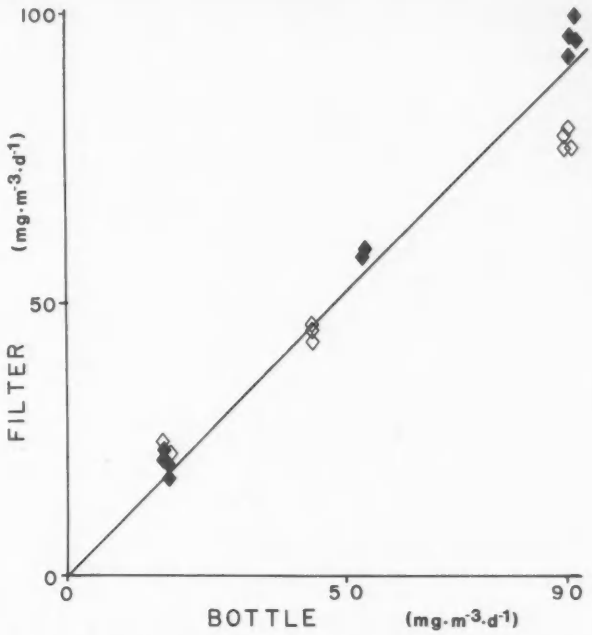

FIG. 1. Comparison of respiration rates measured using the filtration technique to concentrate samples of 250 mL (◆) and 500 mL (◇) with rates measured by incubating unconcentrated water samples for 24 h. The line is the 1:1 line. The slope of the rates measured with the filtration technique regressed against the rates measured by incubating unfiltered samples is not statistically different from 1.0 (t-test, $p > 0.05$, $N = 19$). However, at high respiration rates (>80 $mg \cdot m^{-3} \cdot d^{-1}$), the 500-mL filtration results in lower activity than expected.

F. H. Rigler, unpubl. data). Here we evaluate the influence of the treatments upon the rate of respiration of samples of hypolimnetic water. Most of the experiments discussed below were conducted with water collected from below the thermocline of Lake Memphremagog, a large, mesotrophic lake located on the international border between Canada and the United States (Ross and Kalff 1975; Cornett and Rigler 1984).

Results and Discussion

Our paired design used one sample bottle both to estimate the initial concentration of oxygen and to correct for any experimentally induced changes in concentration. While this design minimizes the influence of sampling artifacts, it assumes that the azide does not influence the results. Sodium azide was chosen because it inhibits respiration, and it is chemically compatible with the Winkler technique of oxygen analysis (American Public Health Association 1965). In preliminary experiments, $HgCl_2$, formalin, and potassium cyanide all influenced the oxygen concentration or interfered with the Winkler analysis.

Three sets of experiments suggest that the addition of azide stopped respiration and did not influence the method in any other way. In the first series of experiments, paired samples were filled simultaneously, and then 200 μL of azide solution was added to one sample and no azide was added to the second. After incubating the bottles at 4°C for 1 d, the samples with and without azide did not have significantly different oxygen

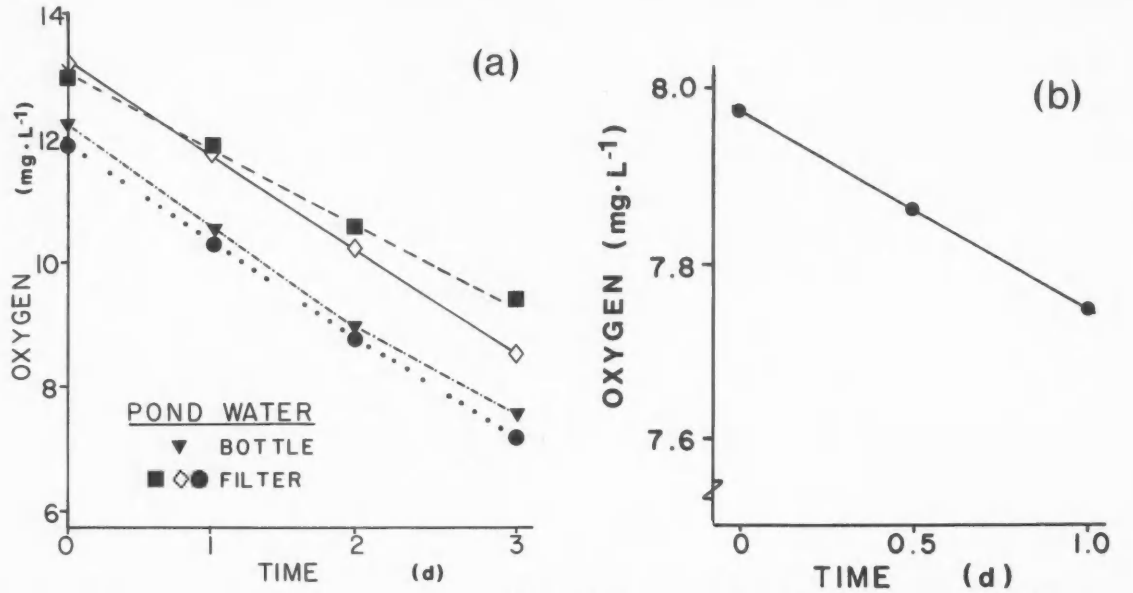

FIG. 2. (a) Changes in oxygen concentration measured over 3 d in 250-mL BOD bottles containing samples of pond water or sterilized pond water plus a filter containing 250 mL of particulates. (b) Changes in oxygen concentration in 60-mL BOD bottles containing 500 mL of filtered hypolimnetic particulate material. Each point is the mean of duplicate or triplicate determinations. The respiration rate of filtered samples did not change during the experiment.

concentrations (Table 1). We suggest that our procedure of incubating the control bottle with azide for 25 h and the respiring bottle with azide for 1 h did not alter the results.

To ensure that azide does stop oxygen consumption, a second test used 12 pairs of sample bottles filled with hypolimnetic water. Then, 250-mL samples of the same water were filtered through 24 filters and 1 filter was added to each bottle. Six pairs of bottles (set A) were treated using the method outlined above. The remaining samples did not receive any azide (set B). Winkler chemicals were added to one sample from each pair of bottles in set B, and the other bottle was returned to the incubator to respire. After 24 h, respiration was stopped by adding azide to the respiring samples in set A and Winkler reagents to the respiring bottles in sets A and B. Rates of respiration for samples in sets A and B were compared in a paired *t*-test. There was no statistically significant difference in respiration in the samples incubated with and without azide (Table 1).

We found that a concentration of 150 mg azide·L^{-1} was sufficient to stop respiration. In the third test, the experiment just described was repeated. Samples in group A received 200 μL of azide solution while 400 μL of azide was added to samples in group B. There was no difference in oxygen consumption or in oxygen concentrations between the two treatments (Table 1). The addition of NaN_3 stopped all respiration and did not interfere with the analysis of oxygen concentrations.

The use of a control sample treated in an identical manner to the respiring sample should ensure that abiotic effects exerted the same influence upon the concentrations of oxygen in both samples. To minimize biotic or microbial contamination of the sample containers and bottles, they were acid washed, or autoclaved, and frozen prior to each experiment. Although the filters were not sterilized, no measurable changes in oxygen concentration were detected between control bottles and bottles with filters incubated in autoclaved water for 1 d. All of these precautions ensured that the sampling methodology did not introduce any artifacts.

Three different types of filters were used to concentrate particulate matter during these experiments. After preliminary testing, Gelman type GA-6™ filters were rejected because they interfered with the Winkler chemistry. Gelman GN-6™ filters and DM-450™ filters both proved useful. There was no significant difference between the respiration rates measured using the two types of filters (*t*-test, $p > 0.5$, $N = 18$). Since DM-450™ filters are composed of polyvinylchloride, which is resistant to microbial attack, these experiments provide further evidence that the filter did not influence the respiration rate of the sample.

In most oligotrophic systems we were unable to obtain respiration rates statistically different from zero unless respiring matter was concentrated upon a filter. However, in Fitch Bay of Lake Memphremagog and Lake Magog, we compared rates of respiration measured using our filtration technique with those measured by incubating unfiltered samples for 1 d (Fig. 1). In 19 replicated comparisons conducted at in situ temperatures (9–13°C), the respiration rates ranged from 20 to 100 mg·m^{-3}·d^{-1}. The rates measured with the two techniques agreed closely. In the poorest comparison, where the filtration technique underestimated the respiration rate by 15%, the samples contained very high concentrations of particulate material. Since the change in oxygen concentration was easily measured without concentrating particulate material, we do not recommend using our technique under these conditions. In all of the other experiments, respiration rates measured using the filtration and bottle techniques agreed within 5%

5% (Fig. 1). We suggest that the filtration did not cause a loss of respiratory activity.

Two sets of the analyses described above were performed using the same water to measure respiration in the unfiltered samples, in water samples concentrated by the filtration of 250 mL, and by the filtration of 500 mL. Comparing respiration rates based upon the filtration of 250 and 500 mL (Fig. 1) showed that at the high rate, respiration was about 20% lower in the 500-mL filtration than rates measured by the 250-mL filtration. The second set of experiments estimated identical low respiration rates (±5%) when 250- and 500-mL water samples were concentrated (Fig. 1). Based on these two analyses we conclude that the different volumes filtered did not influence the measured respiration rate by more than 20%.

Harris and Lott (1973) and Harris and Piccinin (1977) also concluded that the concentration of algal samples by centrifugation or low-pressure filtration did not alter the metabolism or microscopic structure of the cells. In our experiments, no attempt was made to determine what fraction of the metabolic activity remained on the filter after it was resuspended in the sample bottle. However, Griffiths et al. (1973) found that more than 70% of the metabolic activity of microbial samples concentrated by reverse filtration (Pomeroy and Johannes 1966) remained on the filter, even after the filter was backwashed. Similar results would be expected from our direct filtrations. The calibration experiments we conducted were more reproducible than similar experiments using the reverse flow technique (Turner 1978). The percentage of the original metabolic activity recovered was also much higher. The higher, more consistent recovery may reflect either the more rapid filtrations used in our experiments or the smaller volumes of water we filtered. Samples that required longer filtration times than the average did not have either systematically higher or lower respiration rates than the mean. The range of filtration times (2–8 min) used to concentrate the planktonic particles from 250 or 500 mL did not influence their metabolism. The recovery and rate of respiration did not change during the period of incubation. Both high rates of respiration measured using pond water (Fig. 2a) and very low rates measured in hypolimnetic waters (Fig. 2b) were constant over periods of 1–3 d.

We have now measured areal hypolimnetic oxygen deficits and seston respiration in the hypolimnetic water column of 12 lakes where respiration rates ranged from 2 to 80 $mg \cdot m^{-3} \cdot d^{-1}$ (R. J. Cornett and F. H. Rigler, unpubl. data). In the 60 sites investigated during 1979 and 1980 (12 lakes and five sampling depths per lake), there was a strong correlation between the two measurements of oxygen consumption. Respiration rates calculated from the filtration experiments were always less than the total rate of oxygen depletion (i.e. water and sediment respiration). Pooling data from more than 400 duplicate experiments, the average coefficient of variance was 24% when 250-mL samples were filtered and 19% when 500-mL samples were concentrated. Our technique is sensitive and provides a simple method of measuring aerobic respiration or decomposition of organic matter in the pelagic zone of oligotrophic freshwater and marine systems.

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Trypsin from Two Strains of Rainbow Trout, *Salmo gairdneri*, is Influenced Differently by Assay and Acclimation Temperature

J. M. McLeese and E. Don Stevens

Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1

McLeese, J. M., and E. D. Stevens. 1986. Trypsin from two strains of rainbow trout, *Salmo gairdneri*, is influenced differently by assay and acclimation temperature. Can. J. Fish. Aquat. Sci. 43: 1664–1667.

Specific activity and kinetic constants of trypsin from the pyloric caeca of two strains of rainbow trout, *Salmo gairdneri*, were measured using α-*N*-benzoyl-DL-arginine-*p*-nitroaniline·HCl. No increase in activity was observed with cold acclimation, suggesting that cold acclimation induces no increase in trypsin concentration. The apparent K_m for the substrate was independent of assay temperature over the physiological range in both strains, probably to maintain high rates of catalysis at higher temperatures when nutrient requirements are high. Strain A trout produced a trypsin with lower affinity on cold acclimation, but Strain B trout did not. The two strains differed in intestinal morphology as well as in the characteristics of their trypsins.

L'activité spécifique et les constantes cinétiques de la trypsine des caecums pyloriques de deux variétés de truites arc-en-ciel (*Salmo gairdneri*) furent déterminées à l'aide de chlorhydrate de α-*N*-benzoyl-DL-arginine-*p*-nitroaniline. Aucune augmentation de l'activité ne fut observée sous acclimatation au froid; il semblerait que cette acclimatation n'induit pas d'augmentation de concentration de la trypsine. Le K_m apparent du substrat était indépendant de la température de titrage dans l'écart physiologique chez les deux variétés, probablement pour conserver des taux élevés de catalyse à des températures plus hautes lorsque les besoins nutritifs sont élevés. Les truites de variété A ont sécrété une trypsine d'une affinité moindre sous acclimatation au froid alors que les truites de variété B n'ont pas été affectées. Les deux variétés montraient une différence dans la morphologie intestinale ainsi que dans les caractéristiques de leurs trypsines.

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Temperature profoundly affects the rates of chemical reactions. Ectothermic organisms such as fish must avoid temperature change or somehow alter their metabolic machinery to maintain levels of activity appropriate for life in the face of temperature change. Strategies of compensating for temperature may be quantitative, achieved by altering the concentration of appropriate enzymes, or qualitative, achieved by altering the catalytic efficiency of the enzyme (Hochachka and Somero 1984).

If thermal compensation of metabolism occurs, it would be predicted that the systems that provide fuel for metabolism also compensate to some degree. Thus, one would expect digestive enzymes to undergo temperature compensation. Indeed, this appears to be the case on an evolutionary scale. Greenland cod, *Gadus ogac*, possess a trypsin with less temperature sensitivity and greater catalytic ability at low temperature than bovine trypsin (Simpson and Haard 1984).

A few workers have also examined short-term temperature effects on fish digestive systems. The quantitative strategy appears to be important. Cold-acclimated brook trout, *Salvelinus fontinalis*, have pepsin specific activities that are higher than those from warm-acclimated fish measured at the same temperature (Owen and Wiggs 1971). Smit (1967) found rates of gastric acid secretion in cold-acclimated brown bullheads, *Ictalurus nebulosus*, higher than in warm-acclimated fish. Smit suggested that greater acid secretion probably increases pepsin activity because of its low optimal pH.

The majority of digestion and all the nutrient uptake occurs in the intestine. It is of interest, therefore, to examine temperature effects on intestinal enzymes. No quantitative changes occurred with temperature acclimation for the two major proteases, trypsin and chymotrypsin, from rainbow trout, *Salmo gairdneri* (McLeese and Stevens 1982). However, the eel, *Anguilla japonica* (Yoshinaka et al. 1985), and *Salmo trutta* and *Tinca tinca* (Hofer et al. 1975b) possess trypsin isozymes, and the possibility exists that changes in isozymes or in the ratio of isozymes may allow for thermal compensation in rainbow trout. Similarly, genetic differences may optimize trypsin for the temperature regime encountered by a population (Hochachka and Somero 1984).

This study estimates the affinity of trypsin isolated from differently acclimated rainbow trout for a synthetic substrate at a series of temperatures to ascertain the sensitivity to temperature and to see if changes in isozymes or isozyme ratios occurred with acclimation. In addition, we examined trypsin from trout obtained from a second hatchery to see if the temperature responses differed from those of the first group.

Materials and Methods

Two strains of hatchery-reared rainbow trout were sampled 1 yr apart, with acclimation occurring in January of each year. The first, Strain A trout, were obtained from Goossen's Trout Farm Ltd. (Otterville, Ontario) and were divided into two groups for acclimation: (a) 24 fish at 10 ± 1°C and (b) 23 fish at 15 ± 1°C. Strain B trout were obtained from the Normandale

TABLE 1. Percentage of the total caecal weight and the total specific activity contributed by the anterior segment (mean of activities at 5, 10, and 15°C assay temperatures, ± SEM) from two strains of rainbow trout.

Acclimation temperature	Weight (anterior section as % of total caecal weight)	Specific activity · mg protein^{-1} (anterior section as % of total)
Strain A		
10°C	38±1.67	56±0.23
15°C	41±2.19	55±0.13
Strain B		
9°C	70±1.09	56±1.11
15°C	72±0.89	50±0.25

Fish Hatchery (Ontario Ministry of Natural Resources, Simcoe, Ontario) and were divided into two groups: (a) 20 fish acclimated to 9 ± 1°C and (b) 20 fish acclimated to 15 ± 1°C. All were fed to satiation three times daily with diet No. 5 of Cho et al. (1974). Average body weights were obtained at the beginning of each trial by weighing each group of fish, whereas individual body weights and caecal weights were obtained at the end of each trial.

Strain A trout were sampled as described in McLeese and Stevens (1982). Strain B trout were deprived of food for 72 h prior to sampling, and were sampled in one morning. Fish were killed by cervical dislocation, weighed, and the section bearing the caeca cut free. This section was divided into two parts: the anterior section was that from which caeca extended ventrally and dorsally, while the posterior section was that from which caeca extended only dorsally. Each portion was frozen by freeze clamping in liquid nitrogen, wrapped in aluminum foil, and stored at −40°C.

Preparation of supernatant from tissue samples and enzyme activity assays were as described in McLeese and Stevens (1982), using a tris buffer of 0.1 mol · L^{-1} containing 0.04 mol $CaCl_2$ · L^{-1}. Estimates of activity and affinity constants used the synthetic substrate α-*N*-benzoyl-DL-arginine-*p*-nitroaniline · HCl (BAPNA) according to the method of Preiser et al. (1975). An assay time for the kinetic measurements of 2 min was chosen, at which time the reaction was linear. Maximum velocity (V_{max}) and the affinity of the enzyme for the substrate (apparent K_m) were calculated from double reciprocal (Lineweaver−Burk) plots. Protein was measured using the modified Lowry method previously described (McLeese and Stevens 1982).

Results

Caecal morphology differed in the two strains of trout (Table 1). The weight of the anterior section as a percentage of total caecal weight was 38−41% in Strain A fish, but 70−72% in Strain B fish.

In Strain A trout, specific activity and V_{max} were greater in the anterior caeca than posterior caeca (anterior 17−23% greater; statistically significant, $P < 0.0001$). In Strain B trout, the specific activity and V_{max} were greater in the anterior caeca, but the difference was not statistically significant. In both trout strains, the apparent K_m values were the same in anterior and posterior caeca. The effects of acclimation and assay temperature were similar in the anterior and posterior caeca. Total activity, the combined activity values from anterior and posterior caeca not normalized to protein concentration, is presented in Table 2 for both hatchery groups. Strain A trout have higher total activity, or total trypsin potential, than Strain B trout.

Acclimation temperature had no statistically significant effect on specific activity or V_{max} of trypsin from Strain A trout in either the anterior caeca or posterior caeca (Fig. 1, 2; Table 3). However, the apparent K_m values from 10°C acclimated trout were greater (20% when assayed at 10°C and 30% when assayed at 15°C) than those from 15°C acclimated trout. The difference was significant (Table 3).

In Strain B trout, acclimation temperature had no statistically significant effect on specific activity, V_{max}, or apparent K_m over the range of physiological assay temperatures (5−25°C) (Fig. 1, 2).

Specific activities are expressed per unit protein and thus may reflect either changes in activity or changes in protein concentration, or both. Protein concentrations in tissue homogenates Strain A trout ranged from 66 to 71 mg · mL^{-1}, while those from Strain B trout ranged from 63 to 69 mg · mL^{-1} (statistically significant between groups, $P < 0.01$). No significant differences were found between anterior and posterior caeca, nor due to acclimation temperature for either group. Although statistically significant, these differences in protein concentration do not account for the differences in specific activity between the two strains of trout.

Increasing the assay temperature increased the specific activity to a maximum at 55°C. The apparent activation energy of trypsin from Strain B trout, calculated from Arrhenius plots over the range 5−15°C, from 9°C acclimated fish was 55375 J · mol^{-1}, while that from 15°C acclimated fish was 56990 J · mol^{-1}. These values are higher than, but not statistically different from, the apparent activation energy of trypsin from Strain A trout (46196 J · mol^{-1} for both acclimation groups). No significant effect of assay temperature on the apparent K_m was observed in Strain A fish from 10 to 15°C (Fig. 1,2), or in Strain B trout from 5 to 25°C (split-plot ANOVA, $F = 1.29$ $P = 0.29$). However, considering only the data at assay temperatures of 10 and 15°C, K_m increased significantly with assay temperature in Strain B trout posterior caeca (Fig. 2) but not in anterior caeca (Fig. 1).

Discussion

Neither specific activity nor protein concentration changed with acclimation temperature, indicating that there was no difference in the storage of trypsin in the pancreas between warm- and cold-acclimated trout. The diffuse nature of the trout pancreas, however, does not allow the determination of secretory rates. Haschemeyer (1968) found that protein synthetic rates in the liver of toadfish, *Opsanus tau*, are increased with cold acclimation, suggesting that this avenue should be pursued before the quantitative strategy can be eliminated as a mechanism.

Strain A trout acclimated to 10°C had higher apparent K_m values than did those acclimated to 15°C. A new isozyme of lower affinity for substrate appears to have been produced. Because trypsin substrate binding is ionic (Kasserra and Laidler 1969), this may be a compensating change against the increased binding strength caused by the effects of decreased temperature on ionic bonds (Hochachka and Somero 1984). No new isozymes were produced by Strain B trout with cold acclimation.

Assay temperature over the physiological range (5−25°C)

TABLE 2. Average total activity (mean ± SEM, n in parentheses) of trypsin from Hatchery 1 and 2 rainbow trout. Total activity is the sum of the activity measured from anterior and posterior sections and thus is a measure of the total amount of stored trypsin.

	Strain A		Strain B	
Assay temperature (°C)	Acclimation temperature (°C)	Total activity (μg PNA)	Acclimation temperature (°C)	Total activity (μg PNA)
10	10	8112±2248 (16)	9	4723±1137 (13)
10	15	9000±2235 (21)	15	5708±2258 (12)
15	10	11156±3066 (16)	9	6838±1692 (12)
15	15	12248±3069 (21)	15	8596±3258 (12)

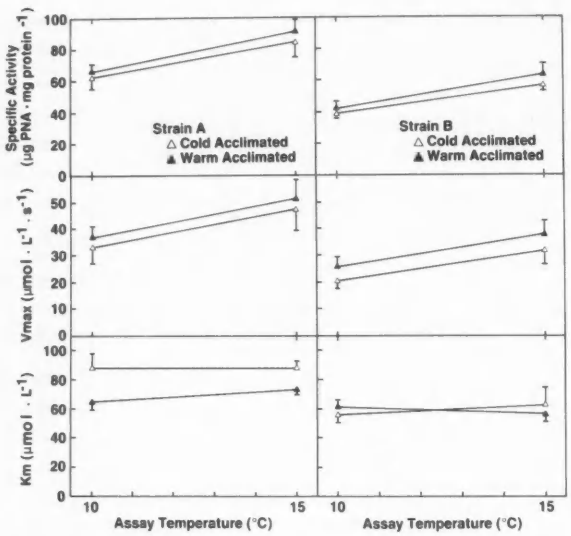

FIG. 1. Differences between Strain A and Strain B trout in the effect of assay temperature at two acclimation temperatures on specific activity, V_{max}, and apparent K_m of trypsin from the anterior caeca. Error bars are ± 1 SEM.

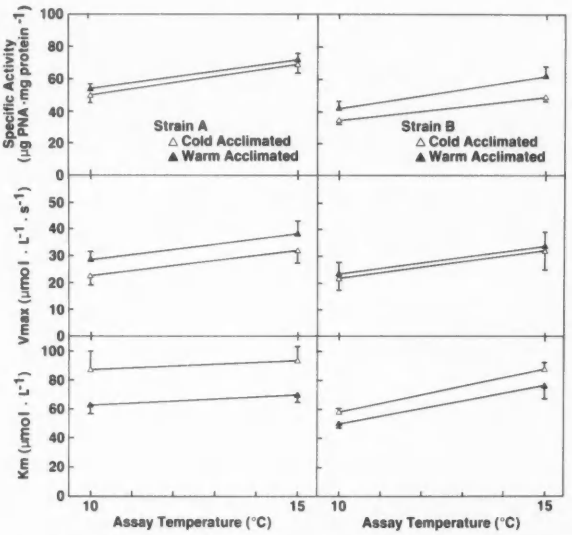

FIG. 2. Differences between Strain A and Strain B trout in the effect of assay temperature at two acclimation temperatures on specific activity, V_{max}, and apparent K_m of trypsin from the posterior caeca. Error bars are ± 1 SEM.

TABLE 3. Difference between two strains of rainbow trout with respect to the significance of the effect of acclimation temperature on the apparent K_m of trypsin. The F value is from analysis of variance acclimation effect and P is the corresponding probability level.

	Strain A		Strain B	
	F	P	F	P
Anterior	7.80	0.009	0.04	0.85
Posterior	6.61	0.017	1.76	0.20
Combined	15.20	0.0002	0.57	0.45

was associated with only small changes in apparent K_m of trypsin from either acclimation group. Independence of apparent K_m from temperature has been found in other fish species (Hofer et al. 1975a; Stevens and McLeese 1984) and in tadpoles, though not in adult amphibians or reptiles (Hofer et al. 1975a). The independence of apparent K_m from temperature resembles that found for certain intracellular enzymes (Yancey and Somero 1978). For intracellular enzymes, such temperature independence allows regulatory control of the enzyme and maintains the K_m within physiological substrate levels. Trypsin, an extracellular enzyme, is under little if any regulatory control once released, and encounters variable substrate levels throughout the course of a meal. It is more likely, therefore, that maintenance of affinity at higher temperatures when binding should be destabilized is an adaptation which allows the animal to hydrolyze substrate rapidly when nutrient demands are high.

The two strains of trout show some differences of note. Strain B trout have approximately 70% of the total caecal weight made up by anterior caeca, compared with approximately 40% in Strain A fish. Since both groups exhibit about 50% of the specific activity in the anterior caeca, then Strain A trout must have more stored trypsin per unit in the anterior caeca than Strain B trout. This difference is probably not physiologically significant, but may reflect different rearing conditions (Bergot et al. 1981) or genetic differences. Strain A fish may have produced a new isozyme when cold acclimated, whereas Strain B trout did not. Total activity was 30–40% higher in Strain A compared with Strain B fish. It is possible that in Strain A fish, the higher total activity helps to compensate for the lower affinity of trypsin during cold acclimation.

It is apparent that the two differently reared groups of trout display different intestinal and pancreatic characteristics, due to environmental and/or genetic factors. The significance and extent of these differences requires further study, both in other systems within the fish and in other groups of trout. Until these variations can be delineated and their importance ascertained, care should be taken in comparisons between differently reared fish and in generalizations to the whole species.

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Susceptibility of Brook Trout (*Salvelinus fontinalis*) Fry to a Liquid Formulation of *Bacillus thuringiensis* serovar. *israelensis* (Teknar®) Used for Blackfly Control

C. Fortin,[1] D. Lapointe, and G. Charpentier[2]

Groupe de Recherche sur les Insectes Piqueurs, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Qué. G9A 5H7

Fortin, C., D. Lapointe, and G. Charpentier. 1986. Susceptibility of brook trout (*Salvelinus fontinalis*) fry to a liquid formulation of *Bacillus thuringiensis* serovar. *israelensis* (Teknar®) used for blackfly control. Can. J. Fish. Aquat. Sci. 43: 1667–1670.

Exposure of brook trout (*Salvelinus fontinalis*) fry to 4500 and 6000 mg Teknar®·L^{-1} for 45 min resulted in 20 and 86.4% mortality, respectively. At concentrations of 3000 mg·L^{-1} and higher, fish became motionless and exhibited variable recovery within 2 h following contact, depending on concentration. Fish exposed to 6000 mg freeze-dried Teknar·L^{-1} were not affected. Forty-eight hours of static exposure resulted in 1.33% mortality at 60 mg·L^{-1}, no mortality at 300 mg·L^{-1}, and 8% mortality for the controls; degradation of water quality may explain this response. Gas chromatographic analysis revealed the presence of 2% xylene in the formulation. Fish fed with chow contaminated with 30% (w/w) Teknar did not show any response. Considering the recovery and the absence of effects in 48-h static tests, the feeding test, and exposure to the freeze-dried formulation, we attribute the observed response to the effect of xylene a preservative agent of the formulation tested.

L'exposition du fretin de l'omble de fontaine (*Salvelinus fontinalis*) à des concentrations de 4500 et 6000 mg·L^{-1} de Teknar® pendant 45 min a résulté en des mortalités finales de 20 et 86.4 %, respectivement. L'exposition aux concentrations de 3000 mg·L^{-1} et plus, a entrainé l'immobilisation des poissons. Un phénomène de récupération, inversement proportionnel à la concentration de Teknar, a été observé. Les poissons exposés a 6000 mg·L^{-1} de Teknar lyophilisé n'ont pas été affectés. En exposition continue de 48-h, sans remplacement d'eau ni aération, les mortalités ont été de 1.33% à 60 mg·L^{-1}, 0% à 300 mg·L^{-1} et 8% pour les témoins; la dégradation de la qualité de l'eau est retenue pour expliquer ces résultats. L'analyse de la formulation, par

[1]Present address: Department of Environmental Biology, University of Guelph, Guelph, Ont. N1G 2W1.

[2]Author to whom reprint request should be addressed.

chromatographie en phase gazeuse, a révélé la présence de 2% de xylène. Les poissons nourris avec de la moulée contaminée au taux de 30% (p/p) de Teknar n'ont montré aucun signe d'intoxication. Considérant la récupération de poissons initialement immobilisés et l'absence d'effets pendant l'exposition statique, le test d'alimentation et l'exposition à la formulation lyophilisée, nous attribuons les résultats obtenus à l'effet toxique du xylène un préservatif présent dans la formulation.

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The discovery of the toxic properties of *Bacillus thuringiensis* serovar. *israelensis* (B.t.i.) on mosquito larvae (Goldberg and Margalit 1977), and subsequent confirmation of its efficacy for control of mosquito (Ali et al. 1981) and blackfly (Colbo and Undeen 1980; Molloy and Jamnback 1981) larvae, constituted a most important step toward efficient and nonpolluting control of biting flies. The main advantage of this material, from an environmental standpoint, is its high selectivity compared with conventional insecticides (Back et al. 1983; Back et al. 1985).

The actual mode of action of this bacterium is not yet completely understood. Crystals of proteic nature, formed during the sporulation of the bacterium, contain a toxin responsible for the observed toxicity (de Barjac 1978). The resulting effects on blackfly larvae were described as a lysis and sloughing of epithelial cells in the mesenteron (Lacey and Federici 1979). Lacey (1985) presented a thorough review of B.t.i. and its applications for mosquito control.

Teknar® (Sandoz, San Diego, CA; lot No. 22291) is a water-based formulation designed for blackfly and mosquito control. The active ingredient is a delta-endotoxin, contained in a protein crystal (parasporal body) of the bacteria. The formulation contains 0.8% active ingredient; the remaining components of the formulation were so called "inert ingredients." A part of this inert fraction was xylene, which is composed of the three isomers of dimethyl benzene. Other monocyclic aromatic hydrocarbons (MAH's), benzene and toluene, are currently used to permeate cell walls of bacteria (American Society for Microbiology 1981). Xylene is believed to break the bacterial cell wall and free the proteic crystal. This property also makes it a good preservative during storage of the product.

On the Canadian Shield, blackfly control operations are conducted at lake outlets during spring and early summer, when brook trout (*Salvelinus fontinalis*) fry are present. Our objective was to determine the susceptibility of brook trout fry to Teknar under actue exposure, to determine the effect of ingestion of this formulation, and then to define the role played by xylene in the observed responses.

Material and Methods

Toxicity tests were conducted in May 1983 and May 1984 in a commercial fish hatchery located at St-Tite (46°45′N, 72°32′W), Québec. The fish were at the parr phase, according to Balon (1975); their average weight was 0.76 g. The fish were randomly divided into lots of 25 and placed in PVC pans (36 × 24 × 14 cm) containing 6 L of continually renewed spring water. Since the B.t.i. is known to act on Diptera only after ingestion, fish were fed during the bioassays. Each tested pan, as well as control pans, received 1 g fish chow·d^{-1} (Purina, size 00) divided among four or five meals.

The following water physicochemical characteristics were monitored during the bioassays: dissolved oxygen (using a 54 ARC oxymeter), pH (Fisher 119, calibrated with an Accumet® 230 A apparatus), conductivity (Myron L DS meter model EP), and temperature (Fisher 14-983-10B thermometer). The observation times for fish response, compared with treatment time (time 0), were −12, −1, 0, 1, 3, 6, 12, 24, 36, and 48 h. At concentrations higher than 300 mg·L^{-1}, the amount of suspended material prevented any observations during contact time. The same schedule was followed for the water quality monitoring except that the 1-h sample was omitted in order to avoid contamination of the monitoring equipment.

Fish response was determined according to the following criteria: (1) unstressed — the fish were swimming normally in the bottom of the pan, with the head toward the current most of the time; (2) stressed — this broad category included fish swimming frantically, laying on their side, having convulsive spasms, or showing only opercle movements; (3) severely stressed — motionless fish, either on the bottom of the pans or floating on the surface.

Since we observed recovery in fish that were motionless, we evaluated a 3-h LC50 following a 45-min contact time. Fish were considered dead when they did not show any respiratory, convulsive, and irritability movements (Sprague 1973).

For flow-through tests the water flow rate was 1 L·min^{-1} for each pan and the volume was maintained at 6 L by overflow holes. At time 0, the water intake was turned off and 1 L of water removed from each pan to premix the formulation. The water containing the formulation was returned to the pans and gently mixed. The water flow was restored after 45 min. Using the guidelines of Sprague (1973), we estimated a contact time of 45 min at full concentration and a 20-min diluting and washing period. The concentrations (and number of replicates) for the flow-through bioassays were 6(4), 60(4), 600(4), 3000(8), 4500(4), and 6000(10) mg Teknar·L^{-1}, 6000(4) mg freeze-dried Teknar·L^{-1} and 6000(4) freeze-dried Teknar with 2% xylene added. This last test was run in order to confirm the role of xylene in the observed mortalities. Xylene alone (120(4) mg·L^{-1}) was also tested. Controls(8) underwent the same manipulations as treated pans.

For static tests, lots of 25 fish were exposed in the same PVC pans without water replacement or aeration for 48 h. The concentrations used were 60(4) and 300(4) mg Teknar·L^{-1} and controls(4).

To determine the effect of ingestion of Teknar on fish, we mixed the formulation with the chow at the rate of 30% (w/w). To avoid differences in texture, taste, or behavior of the contaminated chow in water, compared with noncontaminated chow, we dried it at room temperature for 6 h. Observations were made during the feeding time to ensure that the contaminated chow was consumed. This test was run with four replicates and lasted 48 h.

To remove the solvents from the formulation, we freeze-dried 500 mL of Teknar using a Virtis freeze-dryer for 24 h. The effect of freeze-drying on B.t.i. crystal activity was measured by bioassay on the fourth instar larvae of *Aedes atropalpus* (Diptera: Culicidae). The freeze-dried Teknar was re-

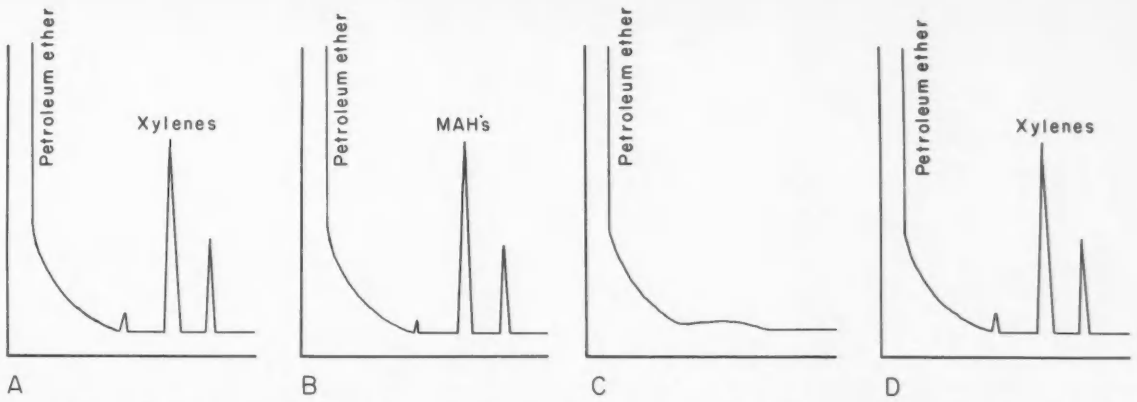

FIG. 1. Chromatograms of petroleum ether extracts of (A) 2% commercial xylene (Fisher X-4) in petroleum ether, (B) MAH's extracted from Teknar, (C) Xylene extracted from freeze-dried Teknar, and (D) Xylene extracted from freeze-dried Teknar with 2% xylene (Fisher X-4) added.

suspended in distilled water and the larvae were exposed following the protocol recommended by the World Health Organization (Brown and Pal 1973).

The presence of xylene in the formulation was determined by extraction and gas chromatographic analysis. Fifty millilitres of Teknar was mixed with 100 mL of petroleum ether (Ligroine 30:60) in a 500-mL glass-stoppered volumetric flask. From the petroleum ether phase (overlayer), 5 μL was injected into a Varian 3700 gas chromatograph equipped with a flame ionization detector. The 3-m stainless steel column (3.2 mm o.d.) contained 15% FFAP on 60/80-mesh Carbowax on Chromosorb W 20 M support. The temperature was programmed to increase from 70 to 200°C at a rate of 50°·min^{-1}. The gas flow rates were as follows: nitrogen (carrier), 30 ± 1; hydrogen, 30 ± 1; air, 300 ± 10 mL·min^{-1}. The temperature of the injector and the detector was 180 and 200°C, respectively.

We identified and quantified the organic solvent extracted by comparison with a standard (xylene, Fisher X-4). The efficacy of the extraction was verified by comparing recoveries from a first and second extraction and from freeze-dried formulation resuspended in distilled water and loaded with 2% xylene.

Results

Fish response to Teknar for flow-through tests was directly related to the concentration of the formulation. No effect, except for a slight excitation during the first 5 min following initial contact, was observed at any concentration less than 3000 mg·L^{-1}. At 3000 mg·L^{-1}, fish appeared stressed for the first 2 h following contact, but all recovered within 3 h. At 4500 mg·L^{-1}, 68% of the fish were stressed, the remaining 32% being severely stressed during the first hour following initial contact. Over a period of 6 h, 80% of the fish had recovered. At 6000 mg·L^{-1}, 92% of the fish were severely stressed during the first hour. After 3 h, 13.6% had recovered. Mortalities (±SD) were 20% ±8.64) at 4500 and 86.4% (±17.3) at 6000 mg·L^{-1}. The 3-h LC50 following a 45-min exposure, determined by probit analysis (Finney 1971), was 5254 mg·L^{-1} (5071−5437, 95% confidence limits). No effect was observed on fish exposed to 6000 mg freeze-dried Teknar·L^{-1}. Fish exposed to 6000 mg freeze-dried Teknar·L^{-1} with 2% xylene added were immediately immobilized and never recovered, resulting in 100% mortality. Fish exposed to xylene alone were stressed during the first minutes of exposure but all recovered after 90 min.

Mortalities (±SD) for fish exposed in static conditions were 1.33% (±0.77) at 60 mg·L^{-1} compared with 8% (±5.66) for the controls. In both cases the mortalities occurred between hours 36 and 48. No mortality was observed at 300 mg·L^{-1}.

The water physicochemical characteristics remained stable during the flow-through tests, the values fluctuating between 90 and 95% oxygen saturation, pH 6.8 and 7.5, and 7 and 9°C, while the conductivity remained stable at 34 μmho·cm^{-1}. During the static test, the dissolved oxygen dropped to 60% saturation after 12 h, the pH dropped to 6.6 after 6 h, the conductivity increased progressively to 62 μmho·cm^{-1}, and the temperature fluctuated between 9 and 11°C.

No effects were observed in fish fed dried contaminated chow. These fish did ingest the contaminated chow with the same behavior as the fish fed with standard chow. Considering the 30% (w/w) contamination of the fish chow, we estimated that each fish ate 0.24 mg Teknar·d^{-1}.

Mosquito larvae exposed to freeze-dried Teknar responded very similarly to those exposed to standard Teknar. Their respective 48-h LC50's were 0.3613 (0.2883−0.4301, 95% confidence limits) and 0.3075 (0.2531−0.3557) mg·L^{-1}.

The formulation used for these tests contained approximately 2% xylene. The efficacy of the extraction and identification methods was confirmed by comparison of chromatograms (Fig. 1). The three peaks are the three isomers of xylene (ortho, para, and meta). No attempt was made to link the peaks with the proper isomer.

Discussion

We suggest that the B.t.i. crystals, or its spore, are not responsible for the observed effects; this conclusion is based on the following observations. The recovery occurring during the 3000, 4500 and 6000 mg·L^{-1} toxicity test would not likely have happened in the case of lysis and sloughing of epithelial cells lining the digestive tract of fish. No such recovery has ever been reported with insects. The absence of effects on fish treated with the freeze-dried Teknar, compared with 86.4% mortality with standard Teknar and with 100% mortality with freeze-dried Teknar with 2% xylene added, and the absence of visible effects during feeding toxicity tests further support the lack of an acutely toxic effect of B.t.i. crystals or spores. The insignificant mortality during the static tests shows that even

for relatively long exposure, B.t.i. did not increase mortalities due to degradation of test conditions.

These observations indicate that B.t.i. crystals or spores are not responsible for the fry mortalities. Xylene present in the formulation is, however, highly suspected. A concentration of 2% xylene in the formulation and formulation concentrations of 3000, 4500, and 6000 mg·L^{-1} corresponds to 60, 90, and 120 mg xylene·L^{-1}, respectively. Benville and Korn (1977) demonstrated that benzene accumulation in the striped bass (*Morone saxatilis*) reached 17 times the water concentration, 1 mg·L^{-1}, in 40 min. The rapid uptake reported by these authors would explain the prompt immobilization that we observed. Considering the high concentrations of xylene and the contact time, uptake of a lethal dose of xylene was probable. The absence of mortality, even if fish presented abnormal behavior, while exposed to xylene alone may be explained by its low water solubility and high volatility. We explain the role of xylene in our results with the complete formulation by its adsorption onto the suspended matter present in the Teknar formulation. This phenomenon could retain xylene in the water column and thereby increase its availability to the fish.

Gills are the primary way of entry of MAH's into fish (Whipple et al. 1981). These authors stated that MAH's are rapidly depurated in fish; however, the detoxification capacity may be exceeded at high concentrations resulting in an accumulation in various organs. The acute toxicity (24-, 48-, and 96-h, LC50's) of xylene and related MAH's (benzene and toluene) to various fish species ranges between 13 and 60 mg·L^{-1} (Brenniman et al. 1976; Stoss and Haines 1979; Ward et al. 1981; Korn and Rice 1981). Morrow et al. (1975) reported that young coho salmon (*Oncorhynchus kisutch*), average weight ranging from 5 to 40 g, died "very quickly" (time not reported) after exposure to 100 mg xylene·L^{-1}. They also reported that fish that survived the first hours of exposure at 10 mg·L^{-1}, or less, survived until the end of the experiment. The fish behavioral reaction reported by these authors was similar to our observations (i.e. erratic swimming, loss of equilibrium followed by immobility). Since the exposure time used by these authors was 96 h, they did not report any recovery.

Our hypothesis of suspended matter acting as a carrier, bringing the toxicant to the gills, may explain the relatively high mortality observed despite the short contact time. The observed effects occurred at much higher concentrations of Teknar and for longer exposure times than would be encountered in blackfly control. During an experimental treatment, Back et al. (1985) used Teknar (same lot No.) at a concentration of 5.5 mg·L^{-1} for an initial contact time of 15 min. They attained 100% control of blackfly larvae at 6°C. The results obtained from these experiments show that Teknar used at normal application rates would not directly affect the fish present in a treated stream. However, effects of any blackfly control operation on insects on which trout or other fish feed have to be considered for a complete impact assessment.

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Instar Increments in Copepod Growth

Alan R. Longhurst

Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

Longhurst, A. R. 1986. Instar increments in copepod growth. Can. J. Fish. Aquat. Sci. 43: 1671–1674.

There is a difference in the growth patterns of large and small copepods as indicated by lengths at each instar for 55 species of copepods from all latitudes. Large species put on a greater proportion of their adult size relatively late in life compared with small species. This confirms an earlier suggestion based on a comparison of only two species.

La détermination des longueurs, à chaque stade de développement, de 55 espèces de copépodes de toutes les latitudes montre l'existence d'un écart entre les modes de croissance des gros et des petits copépodes. Comparativement aux petites espèces, les grosses espèces atteignent la taille adulte relativement tard au cours de leur vie. Cela confirme une hypothèse déjà formulée qui reposait sur la comparaison de seulement deux espèces.

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Copepods, like all other arthropods, grow by size increments at each ecdysis, and it is these increments, together with the duration of each instar, that determine the pattern of their growth. Considering the relative importance of copepods in plankton dynamics, it is surprising that neither of these growth characteristics is comprehensively described; in fact, uncertainty concerning their quantification has clouded recent discussion of how to estimate copepod production (e.g. Tremblay and Roff 1983; McLaren and Corkett 1984).

There have been attemps to derive a comprehensive model for instar duration from analysis of *Acartia* growth, stated as the "isochronal rule" (Miller et al. 1977), although it is clear from later studies of additional species (Landry 1983) that instar duration is not uniform even in species producing several generations during one growth season. Further, in species or stocks having ontogenetic migrations or periods of diapause, some growth stages extend for relatively very long periods; for some arctic copepods, it has been suggested that diapause may even extend over more than 1 yr (e.g. Dawson 1978).

Landry's study appears to establish a sensible first approximation to a comprehensive model of instar duration, capable of being modified by diapause, but we still lack a similar approximation for increments at each ecdysis. It is the purpose of this note to present an analysis of this fundamental characteristic of copepod growth: since copepods, apparently like all crustacea, do not entirely fill the cavity of their new, larger exoskeleton with body tissue after each ecdysis, it may be argued that instar lengths are not really a useful characteristic of their growth trajectories. However, length is very much more rapidly measured than weight, and since Klein Breteler et al. (1982) have shown that length and weight are directly related for several species throughout all larval stages, we can indeed use lengths as a useful indication of the time-course of organic growth. We must remember, however, that length more properly represents mid-instar, rather than initial or terminal weights.

Results and Discussion

The data presented in this short note come entirely from published sources, as indicated in Table 1, which presents lengths at instar for 55 species or populations of cyclopoid and calanoid copepods from both warm and cool oceans. For 20 species, lengths for all instars from the first nauplius to the adult are available, while for the remainder there are only copepodite lengths. This body of data probably represents copepods quite well, and the distribution between large and small species may also reflect the distribution of large and small species in nature. Examination of comparable data sets for weight (e.g. those of Shmeleva 1965) shows these to be much more variable, as indeed they must be unless growth in weight were as discontinuous as growth in length. For this reason, and for questions of availability, the present analysis is based on length data.

Figure 1 presents the descriptive statistics of larval instars for all 55 species in terms of their relative approach to the adult size, and an indication of the relative growth in length achieved at each instar. Relatively large growth increments are achieved at each instar during both the early nauplii and copepodites, and between the last nauplius and the first copepodite. Examination of the growth trajectories of species for which data are available for all larval stages suggests that, although there is much variation, the trajectories leading to the larger adults differentiate from the remainder only during the middle copepodite stages. This appears to reflect the results of Ivanova (1973), who showed that naupliar growth could be described by an exponential function and copepodite growth by a power function.

Figure 2 shows the regression of the growth increment from C3 to C4 (as a percentage of C3) against adult size; the line fitted by least squares shows a positive correlation between relative increase in length at this ecdysis and the final adult size ($r = 0.677$); the value of r^2 suggests that almost half the total variability in relative growth increment is accounted for by adult size ($P < 0.0001$). The growth increment at this ecdysis is described by $y = 0.057x + 0.182$, as a function of adult size. Finally, Fig. 3 shows that there is also a general correlation between the size of early nauplii and their final adult size, while Fig. 4 demonstrates that there is no correlation between rela-

TABLE 1. Instar lengths (mm) of copepods, with data sources used in this study.

Species	N1	N2	N3	N4	N5	N6	C1	C
Euchaeta norvegicus	0.55	0.60	0.64	0.68	0.73	0.78	1.20	1.7
Euchaeta japonica	0.59	0.64	0.65	0.69	0.80	0.87	1.20	1.6
Eucalanus bungii	0.22	0.29	0.49	0.66	0.82	0.97	1.45	2.1
Pleuromamma xiphias								1.1
Calanus tonsus							0.95	1.3
Euchirella messinensis								
Epilabidocera amphitrite	0.19	0.26	0.34	0.40	0.48	0.56	0.79	1.0
Calanus gracilis							1.11	1.3
Pontella meadi	0.23	0.38	0.48	0.62	0.79	0.91	1.11	1.2
Euchaeta hebes							0.85	1.1
Calanus finmarchicus	0.22	0.27	0.40	0.48	0.55	0.61	0.94	1.2
Pleuromamma abdominalis								
Chiridius gracilis							0.58	0.8
Metridia lucens	0.11	0.15	0.18	0.19	0.22	0.29	0.57	0.7
Pontellopsis villosa								
Haloptilus longicornis								
Labidocera aestiva	0.15	0.21	0.30	0.33	0.40	0.47	0.68	0.7
Phaenna spinifera								
Calanus tenuis							0.64	0.8
Heterorhabdus papilliger								
Eucalanus pileatus	0.25	0.30	0.37	0.41	0.52	0.64	0.82	1.1
Pleuromamma gracilis							0.54	0.7
Centropages violaceus							0.38	0.6
Lucicutia flavicornis							0.34	0.4
Nannocalanus minor							0.54	0.7
Aetideus armatus								0.7
Pseudocalanus elongataus	0.18	0.19	0.26	0.33	0.38	0.44	0.46	0.6
Temora longicornis	0.11	0.16	0.21	0.26	0.32	0.38	0.43	0.5
Clausocalanus brevipes							0.42	0.5
Clausocalanus laticeps							0.42	0.5
Centropages hamatus							0.42	0.5
Centropages typicus							0.37	0.4
Acartia clausi	0.12	0.14	0.16	0.19	0.23	0.28	0.43	0.5
Temora stylifera							0.38	0.5
Clausocalanus arcuicornis							0.34	0.4
Centropages typicus	0.11	0.15	0.18	0.19	0.22	0.29	0.29	0.3
Ctenocalanus vanus							0.41	0.6
Ctenocalanus citer							0.41	0.5
Mecynocera clausi								0.3
Clausocalanus furcatus							0.31	0.4
Corycaeus affinis	0.07	0.10	0.11	0.13	0.16	0.19	0.29	0.3
Scolecithrix sp.								0.3
Paracalanus parvus	0.09	0.10	0.16	0.21	0.24	0.25	0.34	0.4
Paracalanus parvus							0.26	0.3
Paracalanus aculeatus							0.26	0.3
Clausocalanus paululus							0.31	0.3
Microcalanus pusillus	0.08	0.09	0.13	0.16	0.18	0.21	0.25	0.3
Oithona similis	0.13	0.15	0.17	0.20	0.23	0.27	0.30	0.3
Calocalanus pavoninus							0.22	0.2
Paracalanus nanus								
Calocalanus contractus								
Calocalanus styliremus								
Paracalanus crassirostris	0.08	0.09	0.12	0.15	0.18	0.20	0.26	0.3
Oithona nana	0.04	0.08	0.10	0.12	0.15	0.19	0.20	0.3
Oithona nana	0.04	0.07	0.09	0.11	0.14	0.17	0.20	0.3

C1	C2	C3	C4	C5	Adult	Location	Reference
.20	1.70	2.30	3.90	5.60	8.10	N Sea	Conway and Minton 1975
.20	1.60	2.40	4.20	5.50	6.30	NE Pac	Campbell 1934
.45	2.11	2.96	3.69	5.03	6.10	NE Pac	Johnson 1937
	1.19	1.59	2.31	3.31	4.84	NE Atl	Ferrari 1985
.95	1.35	1.80	2.90	3.75	4.75	NE Pac	Campbell 1934
		2.10	2.75	3.61	4.54	Med Sea	Shmeleva 1965
.79	1.08	1.46	2.29	3.21	3.60	NE Pac	Johnson 1934
.11	1.36	1.73	2.13	2.77	3.40	Med Sea	Shmeleva 1965
.11	1.29	1.62	1.97	2.86	3.30	NE Atl	Gibson and Grice 1976
.85	1.11	1.42	1.86	2.69	3.10	Med Sea	Shmeleva 1965
.94	1.25	1.65	2.10	2.48	3.08	N Sea	Conway and Minton 1975
		1.37	1.81	2.58	3.01	Med Sea	Shmeleva 1965
.58	0.87	1.16	1.61	2.21	2.89	Gulf St. Lawr	McLellan and Shih 1974
.57	0.76	0.98	1.32	1.94	2.49	N Sea	Conway and Minton 1975
		1.14	1.70	1.99	2.28	Med Sea	Shmeleva 1965
		1.24	1.48	1.65	2.21	Med Sea	Shmeleva 1965
.68	0.79	1.04	1.36	1.63	2.13	NE Atl	Gibson and Grice 1977
		0.96	1.32	1.71	2.13	Med Sea	Shmeleva 1965
.64	0.82	1.09	1.36	1.74	2.07	Med Sea	Shmeleva 1965
		0.74	1.03	1.45	1.96	Med Sea	Shmeleva 1965
.82	1.12	1.28	1.48	1.65	1.95	Trop Atl	Björnberg 1967
.54	0.71	0.86	1.10	1.46	1.95	Med Sea	Shmeleva 1965
.38	0.60	0.74	1.10	1.45	1.83	Med Sea	Shmeleva 1965
.34	0.42	0.60	0.78	1.49	1.78	Med Sea	Shmeleva 1965
.54	0.77	1.01	1.22	1.53	1.75	Med Sea	Shmeleva 1965
	0.75	0.89	1.16	1.39	1.65	Med Sea	Shmeleva 1965
.46	0.64	0.71	0.80	1.05	1.55	N Sea	Conway and Minton 1975
.43	0.51	0.65	0.88	1.19	1.54	N Sea	Conway and Minton 1975
.42	0.56	0.75	0.95	1.27	1.48	NW Atl	Heron and Bowman 1971
.42	0.56	0.71	0.91	1.26	1.47	NW atl	Heron and Bowman 1971
.42	0.53	0.72	0.87	1.23	1.44	N Sea	Conway and Minton 1975
.37	0.48	0.63	0.84	1.09	1.43	Med Sea	Shmeleva 1965
.43	0.57	0.73	0.96	1.13	1.43	N Sea	Conway and Minton 1975
.38	0.55	0.72	0.81	0.98	1.36	Med Sea	Shmeleva 1965
.34	0.49	0.65	0.84	1.06	1.29	Med Sea	Shmeleva 1965
.29	0.38	0.50	0.65	0.88	1.20	N Sea	Conway and Minton 1975
.41	0.61	0.69	0.92	1.12	1.19	Med Sea	Shmeleva 1965
.41	0.55	0.72	0.85	0.98	1.17	NW Atl	Heron and Bowman 1971
	0.38	0.62	0.78	0.98	1.05	Med Sea	Shmeleva 1965
.31	0.43	0.62	0.77	0.91	0.97	Med Sea	Shmeleva 1965
.29	0.37	0.42	0.55	0.74	0.94	NE Pac	Gibson and Grice 1978
	0.50	0.63	0.71	0.84	0.94	Med Sea	Shmeleva 1965
.34	0.45	0.53	0.64	0.80	0.92	N Sea	Conway and Minton 1975
.26	0.31	0.41	0.53	0.79	0.81	Med Sea	Shmeleva 1965
.26	0.37	0.44	0.52	0.59	0.77	Med Sea	Shmeleva 1965
.31	0.38	0.44	0.51	0.59	0.69	Med Sea	Shmeleva 1965
.25	0.36	0.40	0.48	0.55	0.66	N Sea	Conway and Minton 1975
.30	0.37	0.45	0.52	0.59	0.66	N Sea	Conway and Minton 1975
.22	0.28	0.33	0.42	0.50	0.61	Med Sea	Shmeleva 1965
		0.31	0.38	0.48	0.61	Med Sea	Shmeleva 1965
		0.37	0.45	0.54	0.60	Med Sea	Shmeleva 1965
		0.37	0.47	0.50	0.58	Med Sea	Shmeleva 1965
.26	0.34	0.40	0.43	0.47	0.56	NE Atl	Lawson and Grice 1970
.20	0.32	0.36	0.48	0.52	0.55	NE Pac	Murphy 1920
.20	0.33	0.38	0.48	0.52	0.55	N Sea	Conway and Minton 1975

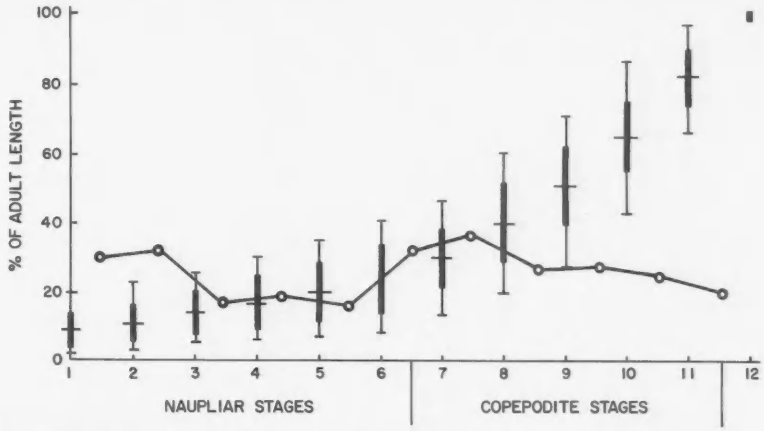

FIG. 1. Mean, standard deviation, and range of larval lengths as percentages of adult female length, and mean relative increment in length at each larval ecdysis. Data for 55 species listed in Table 1.

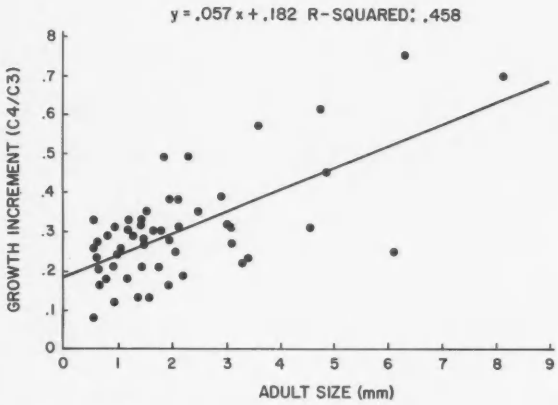

FIG. 2. Regression of relative growth increment from C3 to C4 (increment relative to C3 length) against length of adult female.

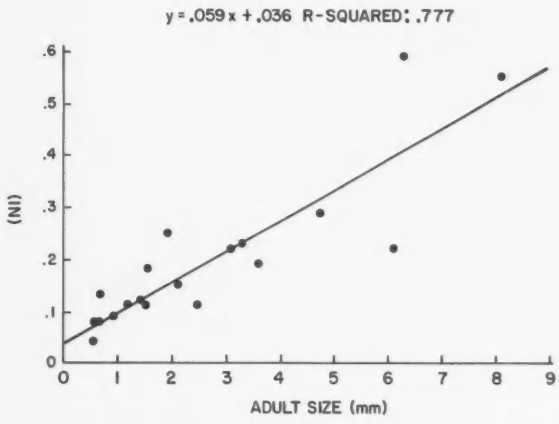

FIG. 3. Regression of lengths of first naupliar stage against length of adult female.

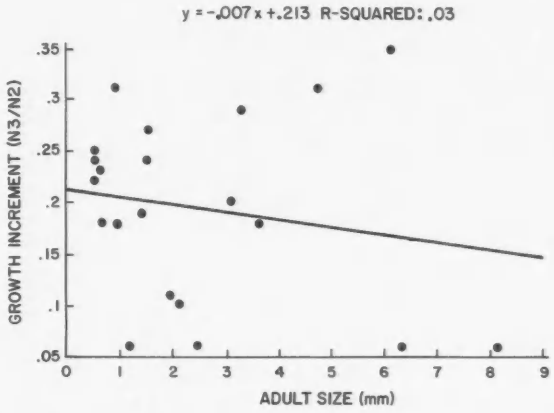

FIG. 4. Regression of relative growth increment from N2 to N3 (increment relative to N2 length) against length of adult female.

tive size increase at mid-naupliar ecdysis and final adult size.

Thus, these data confirm the suggestion of McLaren and Corkett (1984), based on only two species, that large species of copepods accumulate more growth in their later instars than small ones. As McLaren (1969) reminds us, "Brooks Law" suggests that a 25% increase in length between instars is a useful rule of thumb for crustaceans, and that this implies an approximate doubling of volume (hence weight) in an isometrically growing arthropod. Figure 1 shows that the values for instar increments are distributed within 10–15% of a mean value of 26.25%. The largest available block of instar weight data is that of Shmeleva (1965); this suggests that for 16 species of copepods the growth in weight is close to a doubling at each ecdysis. Mean weight increments are C1–C2 = 95%, C2–C3 = 84%, C3–C4 = 112%, C4–C5 = 76%, and C5–Adult = 71%.

The relative length increments (Fig. 1) characteristic of each instar may also be interpreted alongside the analysis of Landry (1983), who found that first-feeding nauplii and C5 instars are no longer than nonfeeding early nauplii and

C1–C4. Clearly, the instars with the longest duration are not those which accomplish the largest increment in length at their terminal ecdysis.

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LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Comment on "Seaweeds, sea urchins, and lobsters: a reappraisal" by R. J. Miller.

Miller (1985) has justifiably pointed out problems with various hypotheses that predators are important in controlling green sea urchin populations. He showed that many of these hypotheses are unsupported and that a decline in predator abundance was probably not the proximal cause of the sea urchin outbreak which occurred in Nova Scotia during the early 1970's. He has done this well, while mentioning the positive contributions of the research which he has criticized. Miller then goes a step further in providing information which he suggests supports the view that predators cannot control green sea urchin populations. Unfortunately the data which he presented in favour of this alternative suggestion is also equivocal. I will point out these problems and examine where we now are regarding predator control of green sea urchin populations.

Miller presented data on field estimates of biomass density for lobsters. Only three of the biomass estimates were made off the Atlantic coast of Nova Scotia. Only one was made in a kelp bed, and as the data were attributed to Miller, unpublished, I must assume that they came from a time *after* lobster stocks had declined. The other two studies (Bernstein and Campbell 1983; Elner and Hamet 1984) were also made during the low period for lobster stocks (see fig. 1 of Miller). Therefore, there are no data available to estimate lobster biomass in a pre-decline kelp bed. What is the lobster biomass in the kelp beds of southwestern Nova Scotia, where lobster landings have remained high? What is the lobster biomass on the Atlantic coast of Nova Scotia now that landings are increasing? The answers to these questions should prove useful in helping to clarify the role of lobster predation in coastal systems in Nova Scotia. The best we can say at present, concerning the role of lobster predation, is that we do not know how important it is, in either kelp beds or barrens. Only tests of appropriate a priori hypotheses based on these questions will allow us to answer them, and thus end the debate.

Miller then provided evidence that fish predation cannot control urchin populations. There is, however, little evidence either way. Wolffish and ocean pout predation were not examined by Miller, as there were no data on their abundance or food in shallow water. The studies reporting low winter flounder predation were from a habitat where urchins were uncommon (Kennedy and Steele (1971) in Long Pond, Newfoundland), or a habitat where the abundance of urchins at a size which flounders could consume was not known (Kennedy and Steele (1971) at Horse Cove, where extremely abundant rugose and undercut corallines and rock crevices provide cryptic habitat for small urchins (pers. obs.) and Wells et al. (1973) in the Bay of Fundy. I dived in the latter area in November 1985, and urchins smaller than 10 mm were extremly rare, possibly because dominant corallines (*Clathromorphum compactum*) were smooth and not undercut, and so provided no refuge for juvenile urchins.) Cunner predation was discounted based on the fact that sea urchin remains constituted >10% of the gut contents in only 3 of 26 cunners. It is unlikely that 26 is an adequate sample size for a species such as the cunner, whose feeding varies with time of day, season, sex, territorial behaviour, and probably whether it is feeding in a group or singly, and on the availability of suitable sized prey (Green et al. 1984; Martel 1982; Martin 1979; pers. obs. 1977–85). As Miller mentioned, sea urchins constituted 15% of the gut contents of cunners in eastern Newfoundland (from Green et al. 1984). Most of those urchins were less than 10 mm (Martel 1982), so the importance of this predation is probably greater than is indicated by the biomass value alone. Winter flounder and cunner are probably the two most abundant species of macrophagous benthic-feeding fish in shallow water rocky habitats off eastern Canada (pers. obs.), and even a small consumption rate per individual might be an important contribution to overall consumption. The size structure of urchin populations in eastern Newfoundland indicates that predation on urchins is high in the <10 mm size group, even in urchin-dominated barrens (Keats et al. 1985). What was the availability, at Miller's study area in Coyle Cove, of urchins within in a size range upon which cunners and winter flounder could feed? The size frequency distributions presented by Mann (1982) suggest that the density of small urchins was probably very low. The best we can say about fish predation at this point is that we have no idea how important it is, in either kelp beds or barrens.

There is another problem with Miller's suggestion that predation is unimportant in "controlling" sea urchin populations. The fact that a predator does not prey on potential prey when the prey availability is low (as in a kelp bed) does not prove that the predator is unimportant in ultimately keeping prey population density low. We must also know the behavioural response of the predator to an increase in recruitment into whatever size class of prey the predator can consume. A relevant question is "Will a *healthy* predator guild dampen the effects of population fluctions in normally incidental prey species by switching their diets to include more of that prey as it becomes more numerically abundant?" There is ample literature to indicate that many predators will do this, so calculations of the "normal" consumption may tell us nothing about the response of a predator to an increase in prey abundance. Since this information has not been determined for lobsters or any other member of the predator guild in Nova Scotia, the implication is that the lobster predation on urchins as calculated by Miller is meaningless in the context within which it has been applied.

The point that I wish to make is that we are back to square one concerning the actual and potential role of predation in controlling green sea urchin populations. The time is ripe for a comprehensive cooperative research program to be developed to formulate appropriate questions, and to design the appropriate experiments to answer them. This could be done with a series of workshops (in a group such as the Working Group on

Animal Macrophyte Interactions which met irregularly during the early 1980's) where the questions are formulated, hypotheses generated, experiments designed, and decisions made as to who will do what. We must start over. We will probably never know what was the proximal cause of the urchin outbreak which occurred off Nova Scotia during the early 1970's, but we can answer in an unequivocal manner some of the important questions which need to be answered to properly manage the coastal ecosystem. I issue this challenge to the Department of Fisheries and Oceans, whose job it is to manage coastal resources, and the academic community in Nova Scotia, whose ongoing marine research alerted us to the problems in the first place — Derek Keats, *Newfoundland Institute for Cold Ocean Science, Memorial University of Newfoundland, St. John's, Nfld. A1B 3X7.* (J8689a)

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Reply to Comment by D. Keats

Keats agrees that evidence favoring predator control of the green sea urchin is not adequate, but he is not satisfied with some of the evidence to the contrary. I argued that the data available on lobsters, crabs, and fish did not support the idea of control, and defined control as a limit to sea urchin grazing sufficient to permit growth of abundant seaweed in suitable habitat.

Keats assumed that biomass densities for Nova Scotia lobsters were from years when abundance was low and, therefore, not adequate for drawing conclusions about predatory impact. As argued in the Perspective, landings should relate to biomass density because of the high exploitation rates. Landings in the statistical areas of the three Nova Scotia studies are compared for the year of the study and a long-term mean (1947–83) as follows: about 50% of the mean, 41 and 94% of the mean (2-yr study), and 195% of the mean (a 35-yr record). Biomass density estimates for 11 additional areas from Long Island Sound to the Gulf of St. Lawrence were also considered in choosing a single value of 10 $g \cdot m^{-2}$ to represent good lobster grounds. This probably comprises the largest series of biomass densities for any marine decapod.

Keats believes there can be no conclusion about fish predation because wolffish and ocean pout stomachs were not examined, and because winter flounder and cunner stomach analyses were on fish from areas where urchins were uncommon or their sizes were unknown. Wolffish and ocean pout were not included because they were never captured and seldom seen in year-round observations at my study site in Coyle Cove. Urchin size frequencies for two sites in question are given in Miller (1985) (fig. 2, June and August) for Coyle Cove and in Vadas et al. (1986) for a site within <1 km of the Bay of Fundy site. I accept Keats' criticism that urchins were uncommon at one Newfoundland study site.

Keats states, "Winter flounder and cunner are probably the two most abundant species of macrophagous benthic-feeding fish in shallow water rocky habitats off eastern Canada"; I am confident that he includes eastern Newfoundland in this assessment. He also lists wolffish and ocean pout as common seasonally in eastern Newfoundland (Keats et al. 1985). In spite of the presence of these predators, urchins are clearly uncontrolled; the sublittoral, rocky habitat of eastern Newfoundland is nearly all classic sea urchin-dominated barrens (Himmelman and Steele 1971; Himmelman 1980; Keats et al. 1985; pers. obs.).

Keats prefaced another comment with, "The fact that a predator does not prey on potential prey when the prey availability is low (as in a kelp bed) does not prove that the predator is unimportant in ultimately keeping prey population density low." On p. 2064 and 2068 of the Perspective, I made the point in reference to lobsters that there are no data for predation rates on scarce or cryptic sea urchins, as they are found in seaweed beds. (This lack of data has not constrained proponents of the predator control hypothesis.) Keats continues by proposing a study on the behavioral response of a healthy predator guild to fluctuating population densities of sea urchins. I wish him success.

Finally, I cannot agree with Keats' contention that we are "back to square one" concerning the problem of predator control of the green sea urchin. There should and will be more studies, but a large body of existing data argues against predator control — R. J. Miller, *Halifax Fisheries Research Laboratory, Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S. B3J 2S7.* (J8689b)

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Comment on Use of the Relative Yield Index to Determine Relationships Between Morphoedaphic Index and Fish Yield by Adams and Olver (1977)

Adams and Olver (1977) attempted to apply Ryder's (1965) morphoedaphic index (MEI) model to data from 70 lakes in Ontario. To obtain significant relationships between the MEI and commercial fish yield, they divided their data in four categories. In doing so they induced an artificial relationship between the two variables. The reasoning is as follows.

The data were subdivided according to the relative yield index (RYI) which relates theoretical yield ($\hat{Y}$) derived from Ryder's (1965) model to the observed yield (Y). The formula for RYI is

$$\text{RYI} = Y/\hat{Y}$$

where $\hat{Y} = 1.4\,(\text{MEI})^{0.45}$ (Ryder 1965). Therefore

$$Y = 1.4\,(\text{RYI})\,(\text{MEI})^{0.45}$$

and

$$\log\,(Y) = 0.146 + \log\,(\text{RYI}) + 0.45\,\log\,(\text{MEI}).$$

Thus, for any given RYI, log (Y) must increase as 0.45 of log (MEI). The upper and lower boundaries for each of Adams and Olver's (1977) four categories are shown in Fig. 1. The 100% RYI boundary gives us Ryder's (1965) model directly, while the others are simply this relationship minus a constant, which will change the intercept of the boundaries but not their slope. The slopes of the relationships for data within the first three categories (RYI = 0–29, 30–49, 50–100%) must therefore tend toward 0.45 depending on the range of MEI included in each category. If the range is short, there will be no significant relationship between MEI and Y (i.e. slope (β) not significantly different from zero). As the range of MEI increases, β will tend more and more toward 0.45. The relationship between MEI and Y for the fourth category (RYI > 100) will depend greatly on the scatter of the data, as it has no imposed upper limit, although it too will be affected by the slope of the lower bound if the range of MEI is sufficiently great.

When considered as a whole, there is no significant relationship between MEI and Y for Adams and Olver's (1977) 70 lakes ($r^2 = 0.03$, $P \geq 0.11$, $F = 2.64$, $N = 70$ (SAS Institute 1982)). We must therefore suggest caution in applying Ryder's (1965) model to these lakes. The subdivision of data into categories by RYI should not be used to determine relationships between the morphoedaphic index and fish yield. As we have shown, this serves only to induce an artificial relationship — M. Robin Anderson, Réjean Fortin, and Sylvain Loranger, *Département des Sciences Biologiques, Université de Québec à Montréal, C.P. 8888, Succursale A, Montréal, Qué. H3C 3P8.* (J8660)

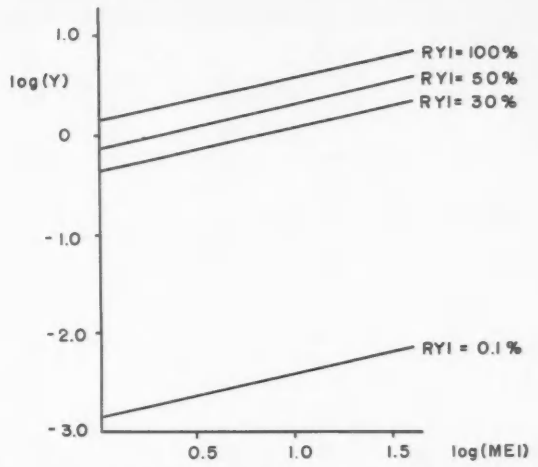

FIG. 1. Upper and lower limits for each relative yield index (RYI) category as defined by Adams and Olver (1977). The axes represent morphoedaphic index (MEI) and commercial fish yield (Y). (Note that we used RYI = 0.1% for the lower boundary of the first category as log (0) does not exist.)

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BOOK REVIEWS / COMPTES RENDUS

Chemical processes in lakes. *Edited by Werner Stumm. John Wiley & Sons, Inc., New York, NY. 1985. 435 p. $59.95 (U.S.).*

Significant advances in the understanding of chemical and biological processes in lakes and the application of that understanding to lake management have occurred in recent years. The specific efforts of researchers are regularly reported in the periodical literature, and workers in these areas are aware of the state of their art. It is, however, somewhat more difficult for the researchers to be fully aware of advances in topics peripheral to their own. Adopting the popular format of a collection of separately authored chapters, Werner Stumm has served the purpose, as stated in his preface "to give an account of current research on chemical processes in lakes" and has provided an opportunity to some leading researchers to synthesize with insight some topics in the field.

The 17 articles cover a range of topics from general conceptual modeling of chemical substances in lakes to application of electrochemical methods to the study of organic solutes and a case study on the Pavin Crater Lake. There is no apparent order to the sequence of chapters, and this is at first disturbing in reading the book cover to cover. However, this organization does provide some variety which makes the sequential reading more enjoyable. There is no reason that the chapters must be read sequentially as each stands on its own. Full comprehension of the topics requires a high level of understanding and background on the part of the reader. The book will likely be of great interest and utility to researchers and graduate students but difficult for those less familiar with limnology or with less training in the physical sciences.

In spite of the variety of topics, several processes in lakes are covered in a number of the chapters. Processes affecting the distribution of trace metals including distribution between dissolved and particular phases, redox-related geochemistry, particle settling, and biological uptake are covered in 10 chapters in different contexts. Sedimentation processes alone are discussed in 10 chapters. Phosphorus dynamics and lake restoration are covered in three chapters. Specific chapters deal with the use of stable carbon isotopes in productivity assessment, the assessment of acidification of aquatic and terrestrial systems, and the chemistry of bog waters.

The strength of the book is in the chapters which synthesize recent research on lakes into process-oriented conceptual and practical models which lead to improved understanding and act as tools for lake management. The first chapter by Dieter Imboden and Rene Schwarzenbach provides a very well organized development of formulation, construction, and application of the box model for the analysis of spatial and temporal distribution of substances in lakes. The discussion is supported by figures and tables carefully chosen to make the equations easier to swallow. The framework established in this chapter is complemented by more specialized "Conceptual Models For Transport at a Redox Boundary" (W. Davison), "Phosphate Interactions at the Sediment Water Interface" (P. Baccini), "The Influence of Coagulation and Sedimentation on the Fate of Particles ..." (C. Omelia), the role of settling particles in metal transfer (L. Sigg), and modeling of the kinetics of chemical processes (J. Morgan and S. Stone). Models have been used in the study of lakes for decades, but Dr. Stumm's collection of examples dramatically underlines the changes which are occurring in the field of limnology. The models are no longer the statistical or graphical fits of the earlier natural history period of limnology. Interactions are described by series of equations taking into account actual physical and chemical conditions and processes. Complex mathematics, physics, and chemistry are no longer sidestepped. Those researchers with classical limnological background may have to make some serious efforts to catch up to these changes. Students in limnology should be aware that sound foundations in mathematics, physics, and chemistry will be essential for future success in the field. This reader hopes that the exploration of the physical and chemical interactions and the discussion of the coupling of biological and geochemical cycles should stimulate other readers to view their own research results in the context of these models.

R. H. HESSLEIN

Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

(R688)

Fish and shellfish pathology. *Edited by A. E. Ellis. Academic Press, London. 1985. xxvi + 412 p. $69.50 (U.S.)*

On September 20–23, 1983, the First International Conference of the European Association of Fish Pathologists was held in Plymouth, England. This book is a collection of the excellent papers presented there. Among these were several timely review articles on selected aspects of fish and shellfish pathology.

In a critical review on the role of pollution as a cause of fish diseases, Möller described the oversimplification inherent in many previously published research efforts purporting to establish causality between aquatic industrial pollutants and the occurrence of chronic diseases in wild fish. He emphasized that there is a primary obligation for scientists to exclude natural phenomena as the major contributing factors of a given disease, before attributing the cause to water pollution. In another article, Ellis presented an overview of the development of vaccines for cultured fish. He and others at the symposium stressed that there were urgent requirements, both for greater theoretical knowledge of the conditions favoring the development of protective immunity in fish and for practical laboratory tests to monitor this immunity. Significantly absent from this conference proceedings were papers enthusiastically predicting the rapid development of new vaccines and bacterins based upon application of routine procedures used in veterinary medicine.

Of particular significance were papers from France and Germany on the vertical transmission of infectious pancreatic necrosis virus (IPNV). Ahne and Negele clearly demonstrated that eyed-eggs of rainbow trout and Arctic charr could easily be infected via water exposure to IPNV. The virus readily attached to the egg shells, where it persisted and remained potentially infectious for more than three weeks. After the eggs had hatched, their virus-contaminated shells remained as a potential source of infection for other fry. That the IPNV is apparently especially stable is substantiated in an accompanying paper by the same authors who describe the survival of IPNV in dried, acetone-treated hypophyseal tissue. This particular report is of special concern to those fish culturists who use pituitary extracts to artificially induce fish to spawn.

Many fish diseases in Europe have not yet reached North America. Two emergent diseases in Atlantic salmon in Norway were described: (1) Hitra disease, a hemorrhagic syndrome of unknown origin, and (2) an unnamed systemic mycosis caused by a newly identified species of *Exophiala*. Aquaculturists interested in importing Atlantic salmon eggs from Europe to North America should be aware of these noncertifiable diseases.

Seven detailed papers were included on bacterial furunculosis of salmonids, but only five articles were contributed on shellfish diseases. Obviously this conference proceedings is not a general text on fish and shellfish pathology, as erroneously implied by the title. Nevertheless, Ellis has done an exemplary job of editing the text in a manner that assures continuity of format and style. A comprehensive index is provided that allows ready access to the information. This book should be useful to practicing fish pathologists and to those fish culturists who are interested in keeping informed of current relevant material on fish diseases.

R. K. KELLY

Department of Fisheries and Oceans
Freshwater Institute
501 University Crescent
Winnipeg, Man. R3T 2N6

(R706)

CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 42, No. 5 — article by G. H. Winters and J. P. Wheeler: *Interaction between stock area, stock abundance, and catchability coefficient.*
Page 995: the equation on Fig. 7 should read "$Y = 191e^{0.033X}$".

VOLUME 42, No. 8 — article by S. P. Millard, J. R. Yearsley, and D. P. Lettenmaier: *Space–time correlation and its effects on methods for detecting aquatic ecological change.*
Page 1397: Equation 9 and definition of its terms should read as follows:

"(9) $$\lambda(x, t) = \mu T\left(\frac{P(x, t)}{K_P + P(x, t)}\right)\left(\frac{Q(t)}{K_Q + Q(t)}\right) + \tilde{\mu}(x, t)$$

where μ = maximum specific growth rate per unit temperature, T = water temperature, . . ."

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

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Assessing juvenile salmonid response to gas supersaturation using a general multivariate dose–response model **J. O. T. Jensen, J. Schnute, and D. F. Alderdice**

Functional maturity and terminal molt of male snow crab, *Chionoecetes opilio* **G. Y. Conan and M. Comeau**

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Analysis of the phytoplankton composition of 95 Labrador lakes, with special reference to natural and anthropogenic acidification **J. C. Earle, H. C. Duthie, and D. A. Scruton**

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Canadian Journal of Fisheries and Aquatic Sciences

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Volume 43, No. 8, August 1986

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Fecundity and egg retention of some sockeye salmon (*Oncorhynchus nerka*) stocks in British Columbia J. I. Manzer and I. Miki **1643–1655**

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